Calcasieu Estuary Remedial Investigation/Feasibility Study (RI/FS): Baseline Ecological Risk Assessment (BERA)

Appendix D: Assessment of Risks to the Aquatic Plant Communities in the Calcasieu Estuary

Prepared For:

CDM Federal Programs Corporation
8140 Walnut Hill Lane, Suite 1000
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Under Contract To:

Mr. John Meyer, Regional Project Manager
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Prepared – October 2002 – By:

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In Association With:

United States Geological Survey
4200 New Haven Road
Columbia, Missouri 65201

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Appendix D. Assessment of Risks to the Aquatic Plant Communities in the Calcasieu Estuary

1.0 Introduction

In response to concerns regarding environmental contamination in the Calcasieu Estuary, a Remedial Investigation/Feasibility Study (RI/FS) is being conducted in the estuary. One of the objectives of the RI/FS is to assess the risks posed by environmental contamination to ecological receptors that inhabit key areas of the Calcasieu Estuary. To meet this objective, a baseline ecological risk assessment (BERA) must be undertaken in accordance with the procedures laid out by the United States Environmental Protection Agency (USEPA) in the *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments* (USEPA 1997). Under the eight-step process described by the USEPA for conducting a BERA, a screening ecological risk assessment (SERA) must be conducted to provide preliminary estimates of exposure and risk.

In 1999, CDM Federal Programs Corporation (CDM) conducted a SERA for the Calcasieu Estuary which concluded that there was a potential risk to ecological receptors inhabiting the estuary from exposure to contaminated sediment and/or surface water (CDM 1999). In September 2001, a Baseline Problem Formulation (BPF; Appendix A; MacDonald *et al.* 2001) was prepared that identified chemicals of potential concern (COPCs) and areas of interest, described the environmental fate and ecological effects of the COPCs, and identified key exposure pathways and receptors at risk in the Estuary. The BPF also led to the development of assessment and measurement endpoints, a conceptual model and a risk analysis plan for the

BERA. Accordingly, the BPF defined the issues that needed to be addressed in the BERA for the Calcasieu Estuary.

One of the important conclusions of the BPF was that aquatic plant communities are likely to be exposed to various COPCs that occur in pore water and surface water. The other groups of aquatic receptors that are addressed in the Calcasieu Estuary BERA include: microbial communities (Appendix C); benthic invertebrate communities (Appendix E2); fish communities (Appendix F1 and F2); avian communities (Appendix H); and, mammalian communities (Appendix I). The COPCs in whole sediments, surface water, and/or pore water that were identified in the BPF included various metals (i.e., copper, chromium, lead, mercury, nickel, and zinc), chlorinated ethanes (i.e., 1,2-dichloroethane and trichloroethane; DCE and TCA), polycyclic aromatic hydrocarbons (PAHs; i.e., 13 parent PAHs and total PAHs), polychlorinated biphenyls (PCBs; i.e., various Aroclor mixtures, PCB congeners, and total PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans [PCDFs; i.e., expressed as tetrachlorodibenzo-*p*-dioxins - toxic equivalents (TCDD-TEQs)], hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), bis(2-ethylhexyl)phthalate (BEHP), carbon disulfide, unionized ammonia, hydrogen sulfide, acetone, and several organochlorine pesticides (i.e., aldrin and dieldrin; see Table A1-7 of Appendix A1).

1.1 Conceptual Model

The conceptual site model represents a particularly important component of the problem formulation process because it enhances the level of understanding regarding the relationships between human activities and ecological receptors at the site under consideration. Specifically, the conceptual site model describes key relationships between stressors and assessment endpoints. In so doing, the conceptual model provides a framework for predicting effects on ecological receptors and a template for generating risk questions and testable hypotheses (USEPA 1997; 1998). The conceptual site model also provides a means of highlighting what is known and what is not known about a site. In this way, the conceptual model provides a basis for identifying data gaps and designing monitoring programs to acquire the information necessary to complete the assessment. Conceptual site models consist of two main elements:

- A set of hypotheses that describe predicted relationships between stressors, exposures, and assessment endpoint responses (along with a rationale for their selection); and,
- Diagrams that illustrate the relationships presented in the risk hypotheses.

The conceptual site model of the Calcasieu Estuary is described in Chapter 7 of the BPF (Appendix A). More specifically, that chapter summarizes the available information on the sources and releases of COPCs, the fate and transport of these substances, the pathways by which ecological receptors are exposed to the COPCs, and the potential effects of these substances on the ecological receptors that occur in the Calcasieu Estuary. In turn, this information was used to develop a series of hypotheses that provide predictions regarding how ecological receptors are exposed to and respond to the COPCs. The conceptual site model, which describes the exposure pathways of greatest interest for aquatic plant communities, was adopted for use in this deterministic risk assessment. Based on the pathways identified in the conceptual model, pore water and surface water are likely to represent the most important routes of exposure for the aquatic plant community. For this reason, other possible exposure pathways (e.g., whole-sediment chemistry) were not evaluated relative to the potential for adverse effects on the aquatic plant communities in the estuary. Because the exposure estimates and chronic toxicity thresholds would be the same for aquatic plants as they are for benthic invertebrates, the risks to aquatic plants associated with exposure to whole sediments would be comparable to those that were estimated for benthic invertebrates (Appendix E2).

1.2 Areas of Concern

The Calcasieu River is one of the largest river systems in southwest Louisiana (LA). From its headwaters in the vicinity of Kisatchie National Forest (in Vernon Parish), the Calcasieu River flows some 260 km to the Gulf of Mexico near Cameron, LA (Figure D-1). While much of the Calcasieu River system is relatively uncontaminated, the portion of the watershed from the saltwater barrier near Lake Charles, LA to the Intercoastal Waterway has undergone extensive industrial development over the past five decades. These developmental activities have resulted in widespread contamination in the estuarine portion of the watershed, particularly in the bayous within the upper portion of the estuary (Curry *et al.* 1997).

In response to public concerns, USEPA is conducting a federally-led RI/FS to assess risks to human health and ecological receptors and to evaluate remedial options for addressing environmental contamination in the Calcasieu Estuary. Based on the results of the SERA, the portion of the Calcasieu Estuary from the saltwater barrier to Moss Lake was identified as the area in which environmental contamination posed the greatest potential risks to ecological receptors and, as such, was designated as the primary study area (CDM 1999). To facilitate the RI/FS, this study area was divided into four sub-areas (termed Areas of Concern; AOC), including:

- Upper Calcasieu River AOC (UCR AOC);
- Bayou Verdine AOC (BV AOC);
- Bayou d'Inde AOC (BI AOC); and,
- Middle Calcasieu River AOC (MCR AOC).

Several reference areas were also identified in the lower estuary and in the vicinity of Sabine National Wildlife Refuge to support the interpretation of the data generated during the RI. As a BERA for the Bayou Verdine has already been completed by Conoco, Inc. and Condea Vista (Entrix, Inc. 2001), ecological risks in the BV AOC were not assessed in this report.

1.3 Chemicals of Potential Concern

The identification of COPCs represents an essential element of the problem formulation process (USEPA 1998). To initiate this process, CDM conducted a SERA of the Calcasieu Estuary in 1999 to assess the potential for adverse biological effects on ecological receptors associated with either direct or indirect exposure to contaminated environmental media in the Calcasieu Estuary (CDM 1999). To support this assessment, historical data on the levels of environmental contaminants in surface water, sediment, and biota were collated and compiled (CDM 1999). Subsequently, the maximum measured concentration of each substance in each media type was compared to the lowest ecological screening value for that substance to facilitate the determination of maximum hazard quotients. These maximum hazard quotients provided a basis for identifying the substances in surface water, sediment, and biota of the estuary that occurred at levels sufficient to potentially affect one or more ecological receptors. These substances were termed COPCs and included: metals; PAHs; PCBs; organochlorine and other pesticides; chlorophenols; chlorinated benzenes; chlorinated ethanes; phthalates; cyanide; and, acetone.

Because the preliminary list of COPCs that emerged from the SERA contained over 100 substances (CDM 1999), it was determined that it required further refinement to assure that only those substances with a relatively high probability of adversely affecting ecological receptors were addressed in further investigations. For this reason, a scoping meeting was convened in Denver, CO in July, 2000 to develop a more focused list of COPCs. The scoping meeting was attended by risk assessors, risk managers, and the USEPA Region VI Ecological Technical Assistance Group (ETAG). Rather than relying on historical data (as was done in the SERA), the participants at this scoping meeting used the results of the Phase I sampling program of the RI to identify the COPCs in the Calcasieu Estuary (Goldberg 2001). For water-borne contaminants, the substances that occurred in unfiltered water samples at total concentrations in excess of the ambient water quality criteria (WQC; i.e., criteria continuous concentrations; CCCs; USEPA 1999) were deemed to be COPCs. For sediment-associated constituents, the substances that occurred in whole sediments at concentrations in excess of the effects range median values (ERMs; Long *et al.* 1995) or comparable sediment quality benchmarks (e.g., probable effect levels PELs; MacDonald *et al.* 1996; CCME 1999) were considered to be COPCs. Based on the results of these evaluations, the scoping meeting participants agreed that the following substances were the primary COPCs in the Calcasieu Estuary:

Water-Borne COPCs

- Metals (copper and mercury);

- 1,2-dichloroethane; and,
- Trichloroethane.

Sediment-Associated COPCs

- Metals (copper, chromium, lead, mercury, nickel, and zinc);
- Polycyclic aromatic hydrocarbons (PAHs; acenaphthene, acenaphthylene, anthracene, fluorene, 2-methylnaphthalene, naphthalene, phenanthrene, benz(a)anthracene, benzo(a)pyrene, chrysene, dibenz(a,h)anthracene, fluoranthene, pyrene, total PAHs, and other PAHs);
- Polychlorinated biphenyls (PCBs);
- Polychlorinated dibenzo-*p*-dioxins (PCDDs), and, polychlorinated dibenzofurans (PCDFs);
- Chlorinated benzenes [hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), and degradation products];
- Phthalates [bis(2-ethylhexyl)phthalate (BEHP)];
- Carbon disulfide;
- Unionized ammonia;
- Hydrogen sulfide;
- Acetone; and,
- Organochlorine pesticides (aldrin and dieldrin).

Because exposure to surface water and pore water represents the principal routes through which the aquatic plant community can be exposed to COPCs, all of the water-borne and sediment-associated substances were identified as COPCs relative to the aquatic plant community.

1.4 Purpose of Appendix

The purpose of this appendix is to provide an evaluation of the risks posed to the aquatic plant communities associated with exposure to COPCs in the Calcasieu Estuary. The aquatic plant communities in freshwater and estuarine ecosystems consist of phytoplankton, periphyton, aquatic macrophytes, and riparian vegetation. Phytoplankton, the small non-vascular aquatic plants that are suspended in the water column, are comprised of several types of algae. While periphyton are also non-vascular aquatic plants, they tend to be larger than the plankton forms of algae and grow on other aquatic plants or on the bottom of the watercourse. Aquatic macrophytes is the general term applied to either large vascular or non-vascular aquatic plants that grow in freshwater, estuarine, and marine systems (including both submergent and emergent aquatic plants). Riparian vegetation is the term that is applied to the vascular aquatic plants that grow along the waters edge.

As primary producers, aquatic plants transform the energy of the sun into organic matter. Aquatic plants represent a primary food source for a variety of aquatic plant-eating invertebrates (i.e., herbivores, which are also known as primary consumers). In addition, aquatic plants provide habitats for a wide variety of species, including aquatic invertebrates. Submergent and emergent aquatic plants provide critical spawning and rearing habitats for many estuarine fish species. Aquatic-dependent wildlife species, such as ducks and geese, also rely on habitats created by aquatic vegetation for reproduction and other life history stages. Hence, aquatic plants represent essential components of aquatic ecosystems.

To date, the ecological assessment work conducted for the Calcasieu Estuary has not characterized the potential risks to the aquatic plant community associated with exposure to COPCs in environmental media. The risk hypotheses laid out in the BPF for the COPCs indicate that many COPCs pose a potential risk to aquatic plants in the estuary from exposure to surface water or to the pore water associated with sediment. This appendix provides an evaluation of the risks posed to the aquatic plant community associated with exposure to the COPCs in the Calcasieu Estuary.

2.0 Methods

A step-wise approach was used to assess the risks to the aquatic plant community posed by exposure to COPCs in the Calcasieu Estuary. The five main steps in this process included:

- Identification of assessment endpoints, risk questions and testable hypotheses, and measurement endpoints;
- Collection, evaluation, and compilation of the relevant information on surface water and sediment quality conditions in the Calcasieu Estuary;
- Assessment of the exposure of aquatic plants to COPCs (i.e., exposure assessment);
- Assessment of the effects of COPCs on aquatic plants (i.e., effects assessment); and,
- Characterization of risks to the aquatic plant community (i.e., risk characterization).

Each of these steps is described in the following sections of this appendix.

2.1 Identification of Assessment Endpoints, Risk Questions, and Measurement Endpoints

An assessment endpoint is an 'explicit expression of the environmental value that is to be protected' (USEPA 1997). The selection of assessment endpoints is an essential element of the overall ecological risk assessment (ERA) process because it provides a means of focusing assessment activities on the key environmental values (e.g., reproduction of sediment-probing birds) that could be adversely affected by exposure to environmental contaminants. Assessment endpoints must be selected based on the ecosystems, communities, and species that occur, have historically occurred, or could potentially occur at the site (USEPA 1997).

A measurement endpoint is defined as 'a measurable ecological characteristic that is related to the valued characteristic that is selected as the assessment endpoint' and it is a measure of biological effects (e.g., mortality, reproduction, growth; USEPA 1997). Measurement endpoints are frequently numerical expressions of observations (e.g., toxicity test results, community diversity measures) that can be compared to similar observations at a control and/or reference site. Such statistical comparisons provide a basis for evaluating the effects that are associated with exposure to a contaminant or group of contaminants at the site under consideration.

To support the identification of key assessment and measurement endpoints for the Calcasieu Estuary BERA, the USEPA convened a BERA workshop in Lake Charles, LA on September 6 and 7, 2000. The workshop participants included representatives of the USEPA, United States Geological Service (USGS), National Oceanic and Atmospheric Administration (NOAA), Louisiana Department of Environmental Quality (LDEQ), United States Fish and Wildlife Service (USFWS) and CDM (hereafter termed the Calcasieu Estuary Ecological Risk Assessment Advisory Group). The workshop was explicitly designed to enable participants to articulate the goals and objectives for the ecosystem (i.e., based on the input that had been provided by the community in a series of public meetings), to assess the status of the knowledge base, to clearly define key issues and concerns, and to identify the COPCs and AOCs in the study area. Importantly, this workshopping approach provided a basis for refining the candidate assessment endpoints that had been proposed based on the results of the SERA (CDM 1999). Additionally, workshop participants identified a suite of measurement endpoints that would provide the information needed for evaluating the status of the assessment endpoints (MacDonald *et al.* 2000).

To be effective, the measurement endpoints must be linked to the assessment endpoints by a series of risk questions. These risk questions, which can be restated as testable hypotheses, describe the specific assumptions about the potential risk to assessment endpoints posed by exposure to COPCs in environmental media. The risk questions that were developed using a combination of professional judgement and information on the potential sources of stressors, stressor characteristics, and actual and predicted ecological effects on the selected assessment endpoints (USEPA 1998). The conceptual model diagrams presented in the BPF provide a visual representation of the risk hypotheses.

2.2 Collection, Evaluation, and Compilation of Relevant Information on Sediment Quality Conditions in the Calcasieu Estuary

Information on the chemical and toxicological characteristics of surface water and/or pore water were collected in two phases, including Phase I and Phase II sampling programs. The methods that were used to collect the samples, quantify the levels of COPCs in various media types, evaluate the toxicity of pore-water samples, evaluate the reliability of the resultant data, and compile the information in a form that would support the BERA are described in the following sections.

Sample Collection - The RI of the Calcasieu Estuary was conducted in two phases. In Phase I of the investigation, surface-water characteristics were evaluated at more than 500 locations throughout the estuary between November, 1999 and March, 2000, based on a stratified random sampling design. The samples collected during this phase of the sampling program were intended to provide the data needed to assess the nature and extent of contamination within the estuary. Full chemistry data were generated for 56 of these samples. The Phase II sampling program was designed to augment the information that was collected in Phase I by providing further information on the nature, severity and areal extent of contamination, assessing the bioavailability of environmental contaminants, evaluating the effects on ecological receptors associated with exposure to contaminants, and filling outstanding data gaps. In Phase II of the investigation, 50 pore-water samples were obtained, again based on a stratified random sampling design. The locations of the sampling sites in the UCR and BV AOCs, BI AOC, MCR AOC, and reference areas are shown in Figures D-2, D-3, D-4a, D-4b, and D-5, respectively.

As indicated above, a stratified random sampling design was utilized in both phases of the RI. More specifically, the estuary was divided into five areas (i.e., UCR AOC, BV AOC, BI AOC, MCR AOC, and reference areas), multiple reaches within each area, and numerous sub-reaches within each reach. Subsequently, the number of samples that were to be collected within each area, reach, and sub-reach was determined based on the size of the area, historic contamination patterns, and other factors. Then, the USEPA Region V Fully Integrated Environmental Location Decision Support (FIELDS) tools were used to randomly select coordinates (i.e., latitude and longitude) for the assigned number of primary sampling stations and alternate sampling stations (i.e., which would sampled if it was not possible to obtain samples from any primary sampling stations). In the field, each sampling station was located with the aid of navigation charts and a Trimble differentially-corrected global positioning system (GPS). Water and sediment samples for obtaining pore water were collected within a 10 meter radius of the designated sampling coordinates.

The methods that were used to collect, handle, and transport the environmental samples collected in the Phase I and Phase II sampling programs are described in CDM (2000a; 2000b; 2000c; 2000d; 2000e). Briefly, surface-water samples were collected with a Kemmerer water sampler, a Van Dorn bottle and/or a peristaltic pump with dedicated tubing. Surficial sediment samples (i.e., the top 10 cm) were collected with a modified large Eckman dredge (23 x 23 cm). At each station, multiple grab samples (up to 10 grabs) were obtained, composited, and homogenized to support the preparation of splits for various chemical analyses and pore water extraction. In accordance with the methods described in CDM (2000e), pore-water samples were obtained by centrifugation of whole sediments at the Marine Environmental Research Center (USGS) in Corpus

Christi, Texas. All pore-water samples were shipped to laboratories in plastic coolers on ice.

Chemical Analyses - Chemical analysis of the environmental samples collected during the Phase I and Phase II sampling programs was conducted at various contract laboratory program (CLP) and subcontract (non-CLP) analytical laboratories, including Quanterra-Severn Trent Laboratories, USEPA Region VI Laboratory, Texas A&M University laboratories, USEPA Region VI CLP laboratories, Olin Contract laboratories, and PPG Industries contract laboratories. Upon receipt at the laboratory, surface-water and pore-water samples were held in coolers until selection for analysis.

The surface-water samples collected in the Phase I sampling program were analyzed by several laboratories. Total metals were quantified using a variety of analytical methods, including E6020, SW6010B, SW6020, and SW7470A. Polycyclic aromatic hydrocarbons and/or other semi-volatile organic compounds (SVOCs) were quantified using one or more of the following methods, SW8260B and SW8270C. Diesel-range organics were measured using SW8015B. Method SW8260B was used to quantify volatile organic compounds. Polychlorinated biphenyls were quantified using SW8082, while pesticides and herbicides were quantified using SW8081A and SW8151A, respectively. Finally, PCDDs and PCDFs were measured using SW8290. A summary of the analyses that were conducted at each analytical laboratory is presented in Table D-1.

The concentrations of COPCs in pore-water samples collected in the Phase II sampling program were determined by three laboratories. The USGS Columbia

Environmental Research Laboratory conducted analyses of total and dissolved metals in pore water using inductively-coupled plasma-mass spectrometry (ICP-MS; May *et al.* 1997). Methyl mercury in pore water was quantified by Texas A&M University using the Trace Element Research Laboratory SOP-9712 and SOP-9024. Method 8270C-SIM, SW8270C, and gas chromatography-mass spectrometry (GCMS; with modifications) were used by American Analytical and Technical Services Ind. (AATS) and Texas A&M University to measure the levels of PAHs in pore water, while SW8081A and SW8081A were used by AATS to quantify PCB (Aroclors) concentrations. Method SW8081A and gas chromatography-electron capture device (GC-ECD; with modifications) were used by AATS and Texas A&M University to measure pesticide concentrations. Finally, congener analysis of PCBs was conducted by Texas A&M University using GC-ECD.

Pore-Water Toxicity - Because aquatic plants represent essential components of the aquatic ecosystem and support many critical ecosystem functions, it is important to evaluate the effects of environmental contaminants on this group of ecological receptors. Aquatic plants can be exposed to environmental contaminants through direct contact with contaminated surface water (i.e., algae, periphyton, macrophytes), through contact with contaminated sediments (i.e., periphyton and macrophytes), and, through contact with contaminated pore water (macrophytes). Although it would be useful to evaluate the effects of environmental contaminants on all three groups of aquatic plants through the various exposure routes, focusing on surface water and pore water provides a means of evaluating the exposure scenarios that are most likely to adversely affect aquatic plants. If adverse effects are not observed or predicted as a result of exposure to surface water or to pore water from contaminated sediments, then it is unlikely that aquatic plants would be adversely affected through other exposure routes.

The effects of sediment-associated contaminants on aquatic plants were evaluated using the results of pore-water toxicity tests. More specifically, the aquatic macrophyte, sea lettuce (*Ulva fasciata*), was used to evaluate the effects of contaminated pore water on aquatic plants. Germination rate, germling length, and cell number of sea lettuce (which were used as surrogates for survival and growth of aquatic plants) were evaluated using the methods described by Hooten and Carr (1998) and Carr *et al.* (2001). Although sea lettuce is primarily a marine species, it is considered to be an appropriate surrogate for freshwater and estuarine aquatic plant species (Hooten and Carr 1998). In this assessment, germination rate, germling length, and cell number of sea lettuce in pore water from Calcasieu Estuary sediments were compared with that of sea lettuce in pore water from reference sediments from the study area.

Pore-water samples were designated as toxic or not toxic using a reference envelope approach. Using this approach, the statistical distribution of the results for samples from reference areas was examined to identify the normal range of responses of sea lettuce exposed to pore water from reference sediments (i.e., which was determined by calculating the 2.5^{th} and 97.5^{th} percentiles of the zoospore germination and growth data following data transformation to achieve normality). Pore-water samples were designated as toxic to aquatic plants if the germination rate, germling length, or cell number of sea lettuce in pore water from Calcasieu Estuary sediments was outside the normal range (i.e., 95% confidence interval) for the pore water from the reference sediments ($p<0.05$). All other samples were designated as not toxic. Although several other procedures could have been used to designate samples as toxic or not toxic [e.g., analysis of variance (ANOVA) compared to control, paired T-tests with control results, minimum significant difference from control; Thursby *et al.* 1997], the reference envelope approach was utilized because it provides a means of evaluating incremental toxicity at test sites when compared to reference sites (Hunt *et al.* 2001). In this way, only the toxicity that is attributable to differences in the characteristics of test and reference samples is considered for the purposes of the BERA. That is, the reference envelope approach provides a basis of determining the toxicity that is attributable primarily to COPC-related factors in the estuary.

Data Validation and Verification - All of the data sets that were generated during the course of the study were critically reviewed to determine their applicability to the assessment of risks to the aquatic plant community in the Calcasieu Estuary. The first step in this process involved validation of all of the surface-water and pore-water chemistry data. Following translation of these data and the pore-water toxicity data into database format, the validated data were then further evaluated to assure the quality of the data used in the risk assessment. This auditing process involved analyses of outliers (i.e., to identify inconsistencies with units) and completeness (i.e., to identify missing samples or missing data); examination of data qualifier fields (i.e., to assure internal consistency in the BERA database), checking of sample identification numbers (to ensure that data were not duplicated). Finally, the data were fully verified against the original data source.

Database Development - To support the compilation and subsequent analysis of the information on water quality and sediment quality conditions in the

Calcasieu Estuary, a relational project database was developed in Microsoft Access format. All of the surface-water chemistry data, pore-water chemistry data, and pore-water toxicity data compiled in the database were georeferenced to facilitate mapping and spatial analysis using geographic information system (GIS)-based applications [i.e., Environmental Systems Research Institute, Inc.'s (ESRI's) ArcView and Spatial Analyst programs]. The database structure made it possible to retrieve data in several ways, including by data type (i.e., chemistry vs. toxicity), by stream reach (i.e., Upper Bayou d'Inde vs. Lower Bayou d'Inde), by sub-reach (i.e., Upper Bayou d'Inde-1 vs. Upper Bayou d'Inde-2), and by date (i.e., Phase I vs. Phase II). As such, the database facilitated a variety of data analyses.

2.3 Assessment of Exposure of Aquatic Plants to Contaminants of Potential Concern

To facilitate assessment of risks to the aquatic plant community, the surface-water and pore-water chemistry data that were collected during the Phase I and Phase II sampling programs were summarized. More specifically, summary statistics were calculated for each AOC of the study area, including the number of samples collected (n), mean and standard deviation, 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles, geometric mean, and range for each COPCs. The mean, standard deviation, and percentile statistics were calculated on $\log_e$ transformed data, based on the assumption that the underlying data were log-normally distributed.

2.4 Assessment of the Effects of Contaminants of Potential Concern on Aquatic Plants

In this assessment, exposure of the aquatic plant community to COPCs was evaluated using information on the concentrations of contaminants in surface water and pore water. Pore water is the water that occupies the spaces between sediment particles and usually accounts for over 50% of the volume of surficial sediments (Power and Chapman 1992). Because sediment-associated contaminants tend to partition into pore water, this medium can represent an important source of exposure to contaminants for sediment-associated organisms (Ingersoll *et al.* 1997). For this reason, pore-water assessments can provide useful information on the potential effects of sediment-associated contaminants, particularly on infaunal species (i.e., those species that routinely reside within the sediment matrix). While such assessments can include several components, pore-water toxicity tests and evaluations of pore-water chemistry represent the central elements of most pore-water assessments (ASTM 2000). Importantly, interpretation of the pore-water chemistry data that is collected in such assessments is dependent on the availability of suitable benchmarks for assessing pore-water quality. Similarly, interpretation of surface water quality data requires the selection of appropriate chronic toxicity thresholds.

Completion of the effects assessment necessitated the compilation of information on the effects on aquatic plant communities associated with exposure to COPCs in these environmental media. Evaluation of the potential effects of COPCs on aquatic plant communities necessitated the selection of two types of chemical benchmarks, including:

- Toxicity thresholds for surface water; and,

- Toxicity thresholds for pore water.

A variety of benchmarks for assessing surface-water and pore-water chemistry are available in the published literature (Appendix E2). Published chronic threshold values for assessing effects of contaminants on aquatic plant communities (i.e., Suter and Tsao 1996) were selected preferentially as toxicity thresholds for surface water and pore water (Table D-2). If a threshold value for aquatic plants was not available in Suter and Tsao (1996) for a particular COPC, then chronic WQC (CCCs) or equivalent values were used to estimate chronic toxicity thresholds for plants (NHDES 1996; NYSDEC 1998; USEPA 1999; LDEQ 2000). The Ecotox thresholds that were developed by USEPA (1996) were used when such WQC were unavailable. Application of the chronic WQC and the Ecotox thresholds for assessing the potential effects of COPCs in pore water on aquatic plants is premised on the assumption that aquatic plants would exhibit a similar range of sensitivities to COPCs as the species that are represented in the underlying toxicological data that was used to generate the various chronic toxicity thresholds.

Pore-water samples with concentrations of one or more COPCs in excess of one or more toxicity threshold listed in Table D-2 were considered to have contaminant concentrations sufficient to adversely affect the survival, growth, and/or reproduction of aquatic plants. Similarly, surface-water samples with concentrations of one or more COPCs in excess of one or more toxicity threshold listed in Table D-2 were considered to have contaminant concentrations sufficient to adversely affect the survival, growth, and/or reproduction of aquatic plants. The comparisons of the levels of COPCs in surface water or pore water to the selected benchmarks was undertaken separately for conventional variables, metals, and organic substances.

2.5 Characterization of Risks to the Aquatic Plant Community

The characterization of the risks to the aquatic plant community consisted of three main steps. First, the nature, severity, and areal extent of risks to the aquatic plant community were evaluated using one or more lines of evidence. Next, the substances that are causing or substantially contributing to effects on the aquatic plant community were identified [i.e., contaminants of concern (COCs)]. Finally, the information on multiple lines of evidence was integrated to evaluate the risks to the aquatic plant community associated with exposure to COCs. The methods that were used in each of these steps of the process are described in the following sections.

Evaluation of the Nature, Severity, and Areal Extent of Risks - In this assessment, three measurement endpoints were used to evaluate the risks posed by COPCs to the aquatic plant community. These lines of evidence included surface-water chemistry, pore-water chemistry, and pore-water toxicity. More specifically, the results of pore-water toxicity tests were used to evaluate the nature of the risks to the aquatic plant community. All three lines of evidence were used to evaluate the magnitude (i.e., severity) of risks to the aquatic plant community. Finally, surface-water chemistry and pore-water chemistry data were used to conduct a preliminary evaluation of the areal extent of risks to the aquatic plant community.

To facilitate characterization of the magnitude and areal extent of risks to the aquatic plant community, risks were classified into three categories for each sample, reach, and AOC. More specifically, risks to aquatic plants were characterized as low, indeterminate, or high, based on the observed magnitude of toxicity and the predicted incidence of toxicity. The following criteria for classifying risks were established based on the guidance that was provided by the

Calcasieu Estuary Ecological Risk Assessment Advisory Group (MacDonald *et al.* 2000; 2001; Table D-3).

Low Risks - ecological risks were classified as low if the effects that were observed or predicted to occur within a sample, reach, or an AOC were similar in frequency and/or magnitude to those for selected references areas (Table D-3). Such effects were considered to be negligible relative to the maintenance of the function of the aquatic plant community within a reach or an AOC. Nevertheless, conditions that require attention may exist within portions of a reach or AOC that was classified as having low risks to the aquatic plant community. Low risks were indicated by:

- 0 to <20% increase in the observed incidence of toxicity (based on % toxic samples) to algae within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata;* endpoint: germination or growth).

- 0 to <20% increase in the predicted incidence of toxicity to aquatic plants within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on comparisons of pore-water or surface-water chemistry to the selected benchmarks for conventional variables, metals, or organic substances).

- 0 to <10% increase in the observed magnitude of toxicity (based on % germination) to algae within a sample, reach, or AOC, relative to the lower 95% prediction limit for the germination that was observed at selected reference sites (i.e., based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*).

Indeterminate Risks - ecological risks were classified as indeterminate if the effects that were observed or predicted to occur within a sample, a reach, or an AOC were moderately higher in frequency and/or magnitude than those for selected references areas (Table D-3). Such effects were considered to be concern relative to the maintenance of the function of the aquatic plant community within a reach or an AOC. Although such risks are nontrivial, decisions regarding remediation at individual locations should consider the costs and ecological effects of remedial actions, the potential for natural restoration, and other relevant factors. It is important to note that low or high risks to the aquatic plant community could exist within portions of a reach or AOC that was classified as posing indeterminate risks. Indeterminate risks were indicated by:

- 20 to 50% increase in the observed incidence of toxicity (based on % of toxic samples) to algae within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata;* endpoint: germination or growth).

- 20 to 50% increase in the predicted incidence of toxicity to aquatic plants within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on comparisons of pore-water or surface-water chemistry to the selected benchmarks for conventional variables, metal, or organic substances).

- 10 to 20% increase in the observed magnitude of toxicity (based on % germination) to algae within a sample, reach, or AOC, relative to the lower 95% prediction limit for the germination that was observed at selected reference sites (i.e., based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*).

High Risks - ecological risks were classified as high if the effects that were observed or predicted to occur within a sample, a reach, or an AOC were substantially higher in frequency and/or magnitude than those for selected references areas (Table D-3). Such effects were considered to be the highest concern relative to the maintenance of the function of the aquatic plant community within a reach or an AOC. Reaches or AOCs so designated represent the highest priority areas for remedial action planning. It is important to note that low or indeterminate risks to the aquatic plant community could exist within portions of a reach or AOC that was classified as posing high risks. Therefore, any remedial actions that are contemplated within such reaches or AOCs should consider the severity and areal extent of the observed and predicted effects. High risks were indicated by:

- >50% increase in the observed incidence of toxicity (based on % toxic samples) to algae within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata;* endpoint: germination or growth).

- >50% increase in the predicted incidence of toxicity to aquatic plants within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on comparisons of pore-water or surface-water chemistry to the selected benchmarks for conventional variables, metals, or organic substances).

- >20% increase in the observed magnitude of toxicity (based on % germination) to algae within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*).

Identification of Contaminants of Concern - The COCs in the Calcasieu Estuary were identified using a step-wise approach. In the first step of this process, the concentrations of COPCs in surface water or pore water in each reach of the estuary (i.e., 95^{th} percentile concentrations) were compared to the concentrations of COPCs in surface water or pore water from the reference areas (i.e., 95^{th} percentile concentrations; the upper limit of background levels). The substances that occurred in the areas of concern at concentrations that were a factor of two or greater than the upper limit of background concentrations (i.e., 95^{th} percentile concentrations) in the reference areas were retained as preliminary COCs in surface water or pore water. Substances were also retained for further assessment if the 95^{th} percentile concentration could not be calculated for the reference area or if the 95^{th} percentile concentration could not be calculated for one or more reaches in an AOC. In both cases, the substance was designated as an uncertain COC. The substances that were designated as preliminary or uncertain COCs were considered to pose potential incremental risks to the aquatic plant community.

In the second step of the process, the estimates of the upper limit of the concentrations of preliminary COCs in surface water or in pore water (i.e., 95th percentile concentrations) in each reach of the study area were compared to the corresponding chemical benchmark (Table D-2). More specifically, the concentrations of preliminary COCs in surface water or pore water were compared to the chronic toxicity thresholds summarized in Table D-2. Substances for which the 95th percentile concentration in surface-water or in pore-water samples in one or more reaches exceeded the selected benchmark were retained as preliminary COCs relative to the aquatic plant community (i.e., the substances for which hazard quotients (HQ) of > 1 were calculated, where HQ = concentration ÷ benchmark). A substance was designated as an uncertain COC if there was no benchmark available for the substance or if the 95th percentile concentration could not be determined for one or more reaches within an AOC (i.e., due to high detection limits).

In the final step of the process, cumulative concentration distribution functions were generated for selected preliminary and uncertain COCs identified in Step 1 and 2 above, using the pore-water chemistry data collected in Phase II. More specifically, the matching pore-water toxicity and pore-water chemistry data were used to develop estuary-wide distribution functions for both toxic and non-toxic samples (i.e., based on the results of the pore-water toxicity tests). Substances were retained as COCs if the cumulative distribution functions for the toxic and non-toxic samples diverged substantially in the upper portion of the concentration range (i.e., the 75th percentile concentration for the effects distribution was a factor of two or more greater than the 75th percentile concentration for the no effects distribution; Long *et al.* 1995).

Integrated Assessment of the Risks to Aquatic Plants using a Weight of Evidence Approach - In this assessment, data from chemical analyses and toxicity tests were used to characterize risks to aquatic plants associated with exposure to COPCs in the Calcasieu Estuary. More specifically, the data on up to three lines of evidence, generated during the RI, were used together to estimate risks to aquatic plants exposed to surface water and/or pore water in the study area. The first step in this process was to calculate a risk score for each measurement endpoint and each line of evidence. Each measurement endpoint was examined to determine if low, indeterminate or high risks were indicated for each sample. A raw risk score of 0, 1, or 2, was assigned to the measurement endpoint based on the risk classification that was assigned (i.e., low, indeterminate, or high, respectively). Next a total evaluation score (TES; i.e., between 1 - low and 3 - high) was calculated to determine the weight that should be placed on the resultant data. The TES was determined by considering a variety of important attributes of the measurement endpoint. By multiplying the magnitude of the risk (raw risk score) by the weight assigned (TES), it was possible to calculate an endpoint risk score of between zero and six for each measurement endpoint. The risk scores for each measurement endpoint were then averaged to calculate an average risk score for each line of evidence for each sample. The information on multiple lines of evidence was then integrated using a simple arithmetic procedure. That is, the average risk score for the various lines of evidence were averaged to generate a final risk score for the assessment endpoint for each sample. Final risk scores of 0 - <2, 2 to 3, and >3 were considered to represent low, indeterminate, and high risks to the aquatic plant community respectively.

3.0 Results and Discussion

The assessment the risks to the aquatic plant community posed by the exposure to COPCs in the Calcasieu Estuary involved several steps. In the first step of the process, the assessment endpoints, risk questions and testable hypotheses, and measurement endpoints were identified (i.e., in the BPF). Next, the relevant information on environmental quality conditions in the Calcasieu Estuary were collected, evaluated, and compiled. Subsequently, the chemical benchmarks for assessing environmental quality conditions were selected, including toxicity thresholds for surface water and pore water. Finally, the risks to the aquatic plant community associated with exposure to surface water and to pore water from the Calcasieu Estuary were assessed. The results of these evaluations are presented in the following sections of this report.

3.1 Assessment Endpoints

The aquatic plant communities in freshwater and estuarine ecosystems consist of phytoplankton, periphyton, aquatic macrophytes, and riparian vegetation. Phytoplankton, the small non-vascular aquatic plants that are suspended in the water column, are comprised of several types of algae. While periphyton are also non-vascular aquatic plants, they tend to be larger than the plankton forms of algae and grow on other aquatic plants or on the bottom of the watercourse. Aquatic macrophytes is the general term applied to either large vascular or non-vascular aquatic plants that grow in freshwater, estuarine, and marine systems (including both submergent and emergent aquatic plants). Riparian vegetation is the term that is applied to the vascular aquatic plants that grow along the waters edge.

There are many different species of algae that can comprise phytoplankton communities, which generally fall into eight main groups. The blue-green algae (cyanophyta) are the most primitive group of algae, with a cell structure like that of bacteria (i.e., the cells lack certain membranous structures, such as nuclear membranes, mitochondria, and chloroplasts; Bell and Woodcock 1968). Blue-green algae can occur in unicellular, filamentous, and colonial forms, many of which are enclosed in gelatinous sheathes. Many species of blue-green algae can utilize nitrogen from the atmosphere as a nutrient (termed nitrogen fixation), which makes them adaptable to a variety of environmental conditions.

Green algae (chlorophyta) encompass a large and diverse group of phytoplankton species that are largely confined to freshwater ecosystems. Green algae can occur as single cells, colonies, or filaments of cells. The chrysophytes are comprised of three groups of algae (diatoms - bacillariophyceae; yellow-green algae - xanthophyceae; golden-brown algae - chrysophyceae) which are linked by a common set of features, including a two-part cell wall, the presence of a flagella, the deposition of silica in the cell wall, and the accumulation of the food reserve, leucosin (Bell and Woodcock 1968). The five other groups of phytoplankton include desmids and dinoflagellates (i.e., pyrrophytes; which are unicellular, flagellate algae), cryptomonads (i.e., cryptophytes; which are typically flagellate algae that grow well under cold, low light conditions), euglenoids (i.e., euglenophytes; which are unicellular, flagellate algae that are only rarely planktonic), brown algae (i.e., phaeophytes), and red algae (i.e., rhodophytes; Bell and Woodcock 1968).

Maples (1987a) developed a checklist of phytoplankton species for the Calcasieu River/Lake complex, including the Calcasieu River, Contraband Bayou, Bayou d'Inde, Bayou Choupique, and Calcasieu Lake. As part of this study, nine stations were sampled monthly over a two year period by towing a 30 µm mesh plankton net for a one minute period. The results of this investigation indicated that the Calcasieu Estuary supports a diverse phytoplankton community, which is comprised of at least 115 taxa representing 61 genera. The most frequently encountered genera included *Asterionella, Chaetoceros, Coscinodiscus, Navicula, Odontella, Pleurosigma, Rhizoslenia, Skeletonema, Thalassiosira, and Thalassiothix* (Maples 1987a). Information on the ecology of phytoplankton communities in Calcasieu Lake is provided by Maples (1987b).

Periphyton is the term that is used to describe the non-vascular aquatic plants that grow on firm substrates, such as sand, gravel, rocks, shells, and aquatic macrophytes (Bell and Woodcock 1968). Like phytoplankton, periphyton are autotrophic organisms that use the energy of the sun to convert inorganic materials (such as carbon, nitrogen, and phosphorus) into organic matter, such as proteins, lipids, and sugars. Periphyton represent an important source of food for benthic and epibenthic invertebrates that feed by grazing on small aquatic plants (Odum 1975). Periphyton communities can be comprised of diverse assemblages of algal species, including members of all of the eight groups of algae that comprise phytoplankton communities (Bell and Woodcock 1968).

Based on the results of studies conducted in the early 1980s, it appears that periphyton communities in the Calcasieu Estuary are comprised largely of diatoms and blue-green algae. Maples (1987c) deployed artificial substrates (i.e., glass slides) for two weeks at 14 stations within the study area (i.e., on a quarterly basis throughout 1984), including five stations in Contraband Bayou, four stations in Bayou d'Inde, and five stations in Choupique Bayou. Taxonomic identification of the periphytic diatoms that accumulated on these substrates indicated that at least 99 taxa representing 30 genera occur in these water bodies. Similar numbers of taxa were observed within each of the three bayous, ranging from 53 taxa in Choupique Bayou to 61 taxa in Contraband Bayou. The most common genera observed in the study area included *Gomphonema, Navicula, Nitzschia, Cyclotella*, and *Bacillaria.*

As part of a related study, Maples (1987d) collected quarterly periphyton samples in 1984 from three bayous in the study area, including Contraband Bayou, Bayou d'Inde, and Choupique Bayou. In this study, periphyton samples were collected by scraping stones, exposed mud flats, and the stems and leaves of littoral vegetation. The results of this investigation showed that blue-green algae represented important components of the periphyton community. In total, 15 blue-green algae taxa were collected in the three bayous, with the most common genera being *Anacystis, Oscillatoria, Microcoleus*, and *Schizothrix*.

Aquatic macrophyte communities are comprised of large vascular and non-vascular aquatic plants that grow in a water body. Aquatic macrophytes can grow under the surface of the water (i.e., submergent aquatic plants, such as milfoil) or emerge from the surface of the water (i.e., emergent aquatic plants, such as bulrushes; Bell and Woodcock 1968). Aquatic macrophytes play several important roles in freshwater and estuarine ecosystems. As autotrophic organisms, aquatic macrophytes can account for much of the primary productivity in aquatic systems, particularly in wetlands and other shallow areas that favor the establishment of marsh plants. In this role, macrophytes represent an important food source for aquatic organisms, either for grazers that can process these aquatic plant materials directly or those species that consume the bacteria that decompose these aquatic plant tissues following their death (Odum 1975). In addition, aquatic macrophytes provide habitats that are utilized by a variety of aquatic invertebrate species, including commercially important species such as shrimp and crabs. These habitats can also represent important spawning and nursery areas for many fish species.

Marsh habitats are particularly important in the Calcasieu Estuary. These habitats can be broken down into four general categories based on the extent of saltwater influence, including saline marsh, brackish marsh, intermediate marsh, and fresh marsh (Perret *et al.* 1970). Saline marshes are located in the areas that are directly exposed to saltwater influences, primarily in the lower portions of the estuary. The dominant emergent macrophytes in saline marshes include oystergrass *(Spartina alterniflora*), glasswort (*Salicornia* sp.), black rush (*Juncus roemerianus*), saltwort (*Batis maritima*), and saltgrass (*Distichlis spicata*). Widgeon grass (*Ruppia maritima*) is the dominant species of submerged vegetation in many saline marshes (Perret *et al.* 1970).

Brackish marsh is generally located adjacent to the saline marsh, but is further removed from the sea rim. This is the predominant type of marsh within the Calcasieu Estuary. Wiregrass (*Spartina patens*), threecorner grass (*Scirpus olneyi*), and coco (*Scirpus robustus*) are the most prevalent aquatic plant species in brackish marshes. The dominant species of submerged vegetation in brackish marshes is typically widgeon grass (*Ruppia maritima;* Perret *et al.* 1970).

Intermediate marshes are found in the lower salinity areas that occur up-gradient of the brackish marshes. The typical emergent macrophyte species in the intermediate marshes include wiregrass (*Spartina patens*), deer pea (*Vigna repens*), bulltongue (*Sagittaria* sp.), wild millet (*Echinochloa walteri*), bullwhip (*Scirpus californicus*), and sawgrass (*Cladium jamaicense*). Wild celery (*Vallisneria* sp.) and spike rush

(Eleocharis sp.) are typically the dominant species of submerged vegetation in intermediate marshes (Perret *et al.* 1970).

Fresh marshes are found in the areas that are not influenced by saltwater intrusion, including those areas upstream of saltwater barriers, at the headwaters of the bayous, and in the vicinity of perched lakes. There are a variety of emergent macrophytes that are typically associated with such fresh marshes, including maiden cane *(Panicum hemitomon*), pennywort (*Hydrocotyl* sp.), pickerelweed (*Pontederia cordata*), alligator weed (*Alternantheria philoxeroides*), bulltongue (*Sagittaria* sp.), and water hyacinth (*Eichhornia crassipes*). The diversity of submergent macrophytes tends to be higher in fresh marshes as compared with the other three marsh types, commonly including fanwort (*Cabomba caroliniana*), coontail (*Ceratophyllum demersum*), bladderwort (*Utricularia vulgaris*), southern naiad (*Najas quadalupensis*), pondweed (*Potamogeton* sp.), and Eurasian milfoil (*Myriophyllum spicatum*; Perret *et al.* 1970).

As primary producers, aquatic plants transform the energy of the sun into organic matter. Aquatic plants represent a primary food source for a variety of aquatic plant-eating invertebrates (i.e., herbivores, which are also known as primary consumers). In addition, aquatic plants provide habitats for a wide variety of species, including aquatic invertebrates. Furthermore, submergent and emergent aquatic plants provide critical spawning and rearing habitats for many estuarine fish species. Many aquatic-dependent wildlife species, such as ducks and geese, rely on habitats created by aquatic vegetation for reproduction and other life history stages. Hence, aquatic plants represent essential components of aquatic ecosystems. As the goal of this assessment is to determine if exposure to COPCs are likely to adversely affect the key functions that are provided by the aquatic plant community, **survival, growth, or**

reproduction of the aquatic plant community were identified as the assessment endpoints for this component of the BERA.

3.2 Measurement Endpoints

A suite of measurement endpoints were selected to provide the information needed to determine if the aquatic plant community is being adversely affected due to exposure to COPCs. First, data on the concentrations of COPCs in pore water or in surface water were used to determine if these media were sufficiently contaminated to adversely affect the survival, growth, or reproduction of aquatic plants in the Calcasieu Estuary. The results of pore-water toxicity tests with the aquatic macrophyte, sea lettuce (*Ulva fasciata*) were also used to evaluate the effects of contaminated pore water on aquatic plants. More specifically, germination rate, germling length, and cell number of sea lettuce (as surrogates for survival, growth, and reproduction of aquatic plants) were evaluated using the methods described by Hooten and Carr (1998). Although sea lettuce is primarily a marine species, it is considered to be an appropriate surrogate for freshwater and estuarine aquatic plant species (Hooten and Carr 1998).

3.3 Risk Questions and Testable Hypotheses

To provide a valid basis for assessing ecological effects, the assessment endpoint need to be linked to the measurement endpoints by a series of risk questions and testable hypotheses. In this study, the investigations to assess the effects of environmental contaminants on the aquatic plant community were designed to answer the following risk questions:

- Are the concentrations of COPCs in surface water from the AOCs in the Calcasieu Estuary greater than the levels of COPCs in surface water from reference areas and greater that the chronic toxicity thresholds for the survival, growth, or reproduction of aquatic plants?
- Are the concentrations of COPCs in pore water from the AOCs in the Calcasieu Estuary greater than the levels of COPCs in pore water from reference areas and greater that the chronic toxicity thresholds for the survival, growth, or reproduction of aquatic plants?
- Is the survival, growth, or reproduction of aquatic plants (as indicated by germination rate, germling length, and cell number of the algae, *Ulva fasciata*) exposed to pore water from Calcasieu Estuary sediments outside the normal range (i.e., 95% confidence interval) for aquatic plants exposed to pore water from reference sediments?

The linkages between the assessment endpoint and the measurement endpoints are articulated in greater detail in the BPF (Appendix A1).

3.4 Exposure of the Aquatic Plant Community to Chemicals of Potential Concern

Exposure is the contact or co-occurrence of a contaminant and a receptor (Suter *et al.* 2000). The exposure assessment is intended to provide an estimate of the magnitude of exposure of receptors to COPCs, over time and space. Both baseline exposure and potential future exposure need to be evaluated during the exposure analysis. For the aquatic plant community, contaminated surface water and pore water were considered to be the principal routes of exposure requiring analysis.

In this investigation, exposure of aquatic plants to COPCs was evaluated for three AOCs and 14 reaches within the study area. Because many of the COPCs considered in this assessment tend to be relatively persistent in sediments, the pore-water chemistry data that were collected during the RI were used to assess both current and near-term future exposure to COPCs in pore water. By comparison, the surface-water chemistry data were used to assess current exposure to COPCs in surface water.

The data on the chemical composition of surface water in the Calcasieu Estuary that were collected during Phase I and Phase II of the RI are presented in Appendix B3. Likewise, the available data on the composition of pore water from Calcasieu Estuary sediments is presented in Appendix B5. The data summaries for each AOC include the number of samples collected (n), mean and standard deviation, geometric mean, 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, and 95^{th} percentiles, and range for each COPCs (See Tables D-4 to D-7 for surface water and D-8 to D-11 for pore water). By comparing the concentrations of COPCs in each AOC to the upper limit of background concentrations [i.e., the 95% upper confidence limit (UCL)] for the reference areas, it is possible to identify the substances that occur at levels that could pose incremental risks to the aquatic plant community, relative to the risk that they pose in reference areas.

Upper Calcasieu River Area of Concern - Within the UCR AOC, the concentrations of chromium (total and dissolved), lead (dissolved), and nickel (total and dissolved), in surface water exceeded the levels that were measured in the reference areas by a factor of two or more (Table D-12). The concentrations of ammonia, copper (total and dissolved), lead (total), mercury (total and dissolved), zinc (total and dissolved), various individual PAHs, Aroclor 1260, aldrin, dieldrin, HCB, 1,1,1-trichloroethane, 1,2-dichloroethane and acetone in the UCR AOC were not elevated relative those in reference areas (Table D-12).

The available pore-water chemistry data indicate that the concentrations of COPCs in pore water from UCR AOC sediments frequently exceed the levels in pore water from reference areas. More specifically, the concentrations of the following substances were measured in pore-water samples at levels a factor of two or more above the 95% UCL for reference areas: hydrogen sulfide; various individual PAHs; certain PCB congeners; and, 1,2,4,5-tetrachlorobenzene (Table D-13). As such, these substances occur in pore water from UCR AOC sediments at levels that could pose an incremental risk to aquatic plants (i.e., relative to the risks that they pose in pore water from reference sediments).

Bayou d'Inde Area of Concern - The results of Phase I and Phase II of the RI indicate that certain COPCs occur in surface water from BI at levels that exceeded the levels that were measured in reference areas by a factor of two or more. More specifically, the concentrations of ammonia, chromium (total and dissolved), copper (dissolved), mercury (total), nickel (total and dissolved), and acetone occurred at elevated levels relative to those that were measured in reference areas (Table D-12). The substances that did not occur in surface water in BI at levels that were elevated relative to those in reference areas are also listed in Table D-12.

The available pore-water chemistry data indicate that the concentrations of COPCs in pore water from BI AOC sediments frequently exceed the levels in pore water from reference sediments. More specifically, the concentrations of the following substances were measured in pore-water samples at levels above the 95% UCL for reference areas: hydrogen sulfide; ammonia; lead (dissolved); nickel (total and dissolved); zinc (total and dissolved); 1;1-biphenyl; various individual PAHs; numerous PCB congeners; total PCBs; aldrin; dieldrin; and, HCB (Table D-13). As such, these substances occur in pore water from BI AOC sediments at levels that could pose an incremental risk to aquatic plants (i.e., relative to the risks that they pose in pore water from reference sediments).

Middle Calcasieu River Area of Concern - Elevated levels of various COPCs (relative to reference areas) were measured in surface-water samples from the MCR AOC. More specifically, the concentrations of chromium (total and dissolved), mercury (total), and nickel (total and dissolved) exceeded levels that were measured in the reference areas by a factor of two or more (Table D-12). The levels of ammonia, copper (total and dissolved), lead (total and dissolved), mercury (dissolved), zinc (total and dissolved), 1,1-biphenyl, various individual PAHs, Aroclor 1260, aldrin, dieldrin, HCB, chlorinated ethanes, and acetone were similar in the UCR AOC and the reference areas (Table D-12).

The available pore-water chemistry data indicate that the concentrations of COPCs in pore water from MCR AOC sediments frequently exceed the levels in pore water from reference sediments. More specifically, the concentrations of the following substances were measured in pore-water samples at levels above the 95% UCL for reference areas: 1,1- biphenyl; all of the individual PAHs; numerous PCB congeners; total PCBs; aldrin; dieldrin; HCB; 1,2,3,4-tetrachlorobenzene; 1,2,4,5-tetrachlorobenzene; and, pentachlorobenzene (Table D-13). As such, these substances occur on pore water from MCR AOC sediments at levels that could pose an incremental risk to aquatic plants (i.e., relative to the risks that they pose in pore water from reference sediments).

Summary - The results of the exposure assessment indicate that a number of COPCs occur in surface-water samples and/or pore-water samples from the Calcasieu Estuary at concentrations in excess of the 95% UCLs for the selected reference areas. These substances that occur in Calcasieu Estuary surface water and/or pore water at elevated levels relative to reference conditions include hydrogen sulfide, ammonia, chromium (total and dissolved), copper (dissolved), lead (dissolved), mercury (total), nickel (total and dissolved), zinc (total and dissolved), all of the individual PAHs, numerous PCB congeners, total PCBs, aldrin, dieldrin, HCB, 1,2,3,4-tetrachlorobenzene, 1,2,4,5-tetrachlorobenzene, pentachlorobenzene, and acetone (Tables D-12 and D-13). As a result, it is concluded that these COPCs occur in surface water and/or pore water from Calcasieu Estuary at levels that could pose incremental risks to aquatic plant communities.

3.5 Effects of Chemicals of Potential Concern on Aquatic Plants

In the analysis of effects, risk assessors determine the nature of toxic effects that are associated with exposure to contaminants and their magnitude as a function of exposure (Suter *et al.* 2000). Information on the effects of environmental contaminants may be acquired from the results of single chemical toxicity tests (e.g., water-only toxicity tests), ambient media toxicity tests (e.g., the results of toxicity tests conducted using pore water collected from the site under investigation), and/or biological surveys (e.g., aquatic plant community assessments). Importantly, the data that are collected during this phase of the assessment should be directly related to the exposure estimates, thereby facilitating characterization of risks to each assessment endpoint.

In this assessment, exposure of the aquatic plant community to COPCs was evaluated using information on the concentrations of contaminants in surface water and pore water. As such, it was necessary to compile information on the effects on aquatic plant communities associated with exposure to COPCs in these environmental media. Table D-2 provides a summary of the benchmarks that were selected for assessing surface-water and pore-water chemistry relative to the potential for adverse effects on aquatic plant communities. Whenever possible, these benchmarks represent the lowest chronic toxicity threshold published for aquatic plants (Suter and Tsao 1996). Otherwise, chronic WQC or equivalent values were selected as the benchmarks for assessing risks to aquatic plants (USEPA 1996; 1999; NHDES 1996; NYSDEC 1998; LDEQ 2000).

3.6 Characterization of Risks to the Aquatic Plant Community

The purpose of risk characterization is to determine if significant effects are occurring or are likely to occur at the site under investigation. In addition, this step of the process is intended to provide the information needed to describe the nature, magnitude, and areal extent of effects on the selected assessment endpoints. Finally, the substances that are causing or substantially contributing to such effects (termed COCs) are identified from the COPCs. This information is generated by integrating the results of the exposure assessment with the results of the effects assessment, with each line of evidence initially considered separately. An evaluation of the uncertainty in the analysis provides a basis for determining the level of confidence that can be placed on these results and for integrating multiple lines of evidence into an overall assessment of risks to the aquatic plant community. In the final step of the process, the various lines of evidence were considered together to establish a weight of evidence for assessing risks to the assessment endpoint under consideration.

To support the objectives of the risk characterization process, the results of Phase I and Phase II of the RI were compiled and used to assess risks to the aquatic plant community associated with exposure to COPCs in surface water and pore water. Three lines of evidence were examined to determine if exposure to environmental media in the Calcasieu Estuary poses significant risks to the aquatic plant community, including surface-water chemistry, pore-water chemistry and pore-water toxicity.

Evaluation of the surface-water chemistry data collected during the Phase I RI indicates that the concentrations of COPCs in samples from the three AOCs, are unlikely to be sufficient to cause or substantially contribute to chronic toxicity to aquatic plants. Based on comparisons to benchmarks, the predicted incidence of toxicity in the three AOCs is 13% (2 of 15 samples), 100% (55 of 55 samples) and 9% (5 of 55), respectively, when the data on the levels of conventional variables, metals, and organic substances are considered (Tables D-14 to D-16). By comparison, the predicted incidence of toxicity to aquatic plants exposed to surface water from reference areas was 0% (n=1), 100% (n=1), and 0% (n=1), respectively, when the three groups of water quality variables were considered for reference areas. Therefore, exposure to surface water is unlikely to adversely affect aquatic plants in the Calcasieu Estuary.

For pore water, the concentrations of hydrogen sulfide or ammonia, metals, and organic substances were sufficient to cause or substantially contribute to chronic toxicity to aquatic plants in 69% (52 of 75), 100% (38 of 38), and 5% (2 of 38), respectively (Tables D-17 to D-19). The predicted incidence of toxicity to aquatic plants in reference areas was substantially lower (21%; n=14) when the concentrations of conventional variables were considered (Table D-17). In contrast, the predicted incidence of toxicity to aquatic plants in reference areas was similar when the concentrations of metals (100%; n=7), and organic substances (0%; n = 7), were considered (Tables D-18 to D-19). Based on the results of 96-h toxicity tests with the alga, *Ulva fasciata* (endpoint: germination or growth), the incidence of toxicity within the three AOCs was 21% (8 of 38), within the three AOCS and 14% (1 of 7) for the reference areas (Table D-20).

When considered together, these three lines of evidence indicate that exposure to surface water or pore water is generally not adversely affecting the survival, growth, and reproduction of aquatic plants in the Calcasieu Estuary. However, elevated levels of certain conventional variables in pore water do pose risks to aquatic plants in the estuary. Accordingly, each of the three AOCs were examined in greater detail to assess the nature, magnitude, and areal extent of risks to aquatic plant communities.

3.6.1 Upper Calcasieu River Area of Concern

The Upper Calcasieu River includes the portion of the watershed from the saltwater barrier to the Highway 210 bridge, a distance of roughly 12 km (or 15 km, including the loop of the river located south of the saltwater barrier). This portion of the river system consists of several readily identifiable water bodies, including the Upper Calcasieu River mainstem from the saltwater barrier to Lake Charles, Lake Charles, Calcasieu Ship Channel from Lake Charles to the Highway 210 bridge, Clooney Island Loop, Contraband Bayou, Coon Island Loop, and Bayou Verdine (Figure D-2). The areas of interest within the UCR AOC include the Clooney Island Loop, Clooney Island Loop barge slip, Coon Island Loop northeast, and Coon Island Loop southwest (MacDonald *et al.* 2001). To facilitate assessment of risks to the aquatic plant community, the UCR AOC was divided into four main reaches, including:

- Upper Calcasieu River - Mainstem and Calcasieu Ship Channel (i.e., from the saltwater barrier to upstream boundary of the BI AOC; Figure D-2);
- Clooney Island Loop (i.e., Figure D-2);
- Contraband Bayou (i.e., from the headwaters to the mouth; Figure D-2);
- Coon Island Loop (Figure D-2).

The risks to the aquatic plant community posed by exposure to surface water or pore water were evaluated for each of these reaches and for the UCR AOC as a whole. Additionally, hot spots with respect to contaminated surface water or pore water were identified when possible.

3.6.1.1 Nature of Effects on the Aquatic Plant Community in the Upper Calcasieu River Area of Concern

In total, data on three measurement endpoints were used to determine if adverse effects on the aquatic plant community were occurring in the UCR AOC in response to exposure to COPCs, including surface-water chemistry, pore-water chemistry, and pore-water toxicity. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to aquatic plant communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring or are likely to be occurring within the study area, and to determine the nature of those effects.

The surface-water chemistry data collected during the Phase I RI were evaluated using chronic toxicity thresholds for aquatic plants (or equivalent benchmarks). None of the samples (n=4) collected in this AOC had hydrogen sulfide or ammonia concentrations sufficient to adversely affect aquatic plants (Table D-14). Similarly, the predicted incidence of toxicity, calculated using data on the levels of metals or organic substances in surface water were not sufficient to result in a higher predicted incidence of toxicity in the UCR AOC than was the case for the reference areas (Tables D-15 and D-16) Therefore, it is apparent that surface-water quality conditions are unlikely to pose a significant risk to the aquatic plant community (i.e., survival, growth, and reproduction are unlikely to be adversely affected by exposure to surface water).

The pore-water chemistry data collected during the Phase II RI were compared to the chronic toxicity thresholds (Tables D-17 to D-19). The results of this evaluation indicate that one or more chronic toxicity thresholds for metals were exceeded in all of the pore-water samples (n=15) collected from the UCR AOC in the Calcasieu Estuary, which is the same as the predicted incidence of toxicity for reference areas (i.e., based on one or more exceedances of the toxicity thresholds for metals in pore water). By comparison, the predicted incidence of toxicity was 62% (18 of 29 samples) when only the concentrations of hydrogen sulfide and ammonia were considered, which is higher than the incidence of toxicity (21%; n = 14) that was predicted for reference areas. When the concentrations of organic substances were considered, the predicted incidence of toxicity in the UCR AOC was 0% (n=15). These data suggest that, at minimum, the concentrations of hydrogen sulfide and/or ammonia in pore water are sufficient to adversely affect the aquatic plant community.

The results of pore-water toxicity tests provide a basis for assessing the risks to aquatic plant communities associated with exposure to COPCs in the Calcasieu Estuary. Overall, three of the 15 pore-water samples (20%) from the UCR AOC were toxic to the alga, *Ulva fasciata*, in 96-h toxicity tests when zoospore germination or growth were considered (Table D-20; Figure D-6). By comparison, the incidence of toxicity was 14% (1 of 7 samples) for algae zoospores exposed to pore water from sediments from reference areas. These data suggest that the survival, growth or reproduction of aquatic plants exposed to pore water from UCR AOC sediments are only infrequently compromised in the AOC.

When considered together, these three lines of evidence indicate that exposure to surface water or pore water is unlikely to pose significant risks to the aquatic plant community (i.e., the survival, growth, and reproduction of aquatic plant community in the UCR AOC are likely to be similar to those observed in reference areas). Therefore, it is concluded that significant effects on the aquatic plant community are generally not occurring in the UCR AOC. Nevertheless, elevated levels of certain COPCs in pore water (i.e., conventional variables) represents a concern at various locations in the UCR AOC.

3.6.1.2 Magnitude of Effects on the Aquatic Plant Community in the Upper Calcasieu River Area of Concern

The magnitude of the effects on aquatic plants in the UCR AOC was evaluated using one line of evidence: pore-water toxicity. Based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata* (i.e., observed magnitude of toxicity), it is apparent that exposure to pore water from UCR AOC sediments generally does not adversely affect the germination of algal zoospores. Of the 15 pore-water samples that were collected from the UCR AOCs, a total of 12 (80%) were found to pose a low risk to algae (i.e., germination rates of *Ulva fasciata* were similar to those observed for samples from reference areas). By comparison, the germination of algal zoospores was reduced by >20% in three of the samples (20%) from the UCR AOC (Table D-21; Figure D-7).

The information on the observed magnitude of toxicity to the alga, *Ulva fasciata*, indicates that exposure to pore water from UCR AOC sediments generally poses a low risk to the aquatic plant community (Table D-21). Nevertheless, the results of this assessment indicate that this AOC has a number of hot spots with respect to pore-water toxicity that may require further assessment and/or remedial action.

3.6.1.3 Preliminary Assessment of the Areal Extent of Effects on the Aquatic Plant Community in the Upper Calcasieu River Area of Concern

A preliminary assessment of the areal extent of adverse effects on aquatic plant communities in the UCR AOC was conducted using the pore-water toxicity data that were collected in Phase II of the RI. To support this evaluation of the spatial distribution of effects, each sample was classified into one of three categories (i.e., low, indeterminate, or high), based on the risk that it posed to aquatic plants (as indicated by the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*). Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial Analyst software. The reaches that were considered in this analysis included the Upper Calcasieu River mainstem reach, Clooney Island Loop reach, Contraband Bayou reach, and Coon Island Loop reach.

Upper Calcasieu River - Mainstem Reach - Pore-water toxicity data are available for a total of four samples from the Upper Calcasieu River mainstem reach of the UCR AOC. The results of these toxicity tests indicate that a pore-water sample from the eastern portion of Lake Charles was toxic to aquatic plants (Figure D-6; Table D-20). Overall, COPCs in pore water are considered to pose a low risk to aquatic plants in this reach of the UCR AOC.

Clooney Island Loop Reach - For the Clooney Island Loop reach of the UCR AOC, pore-water toxicity data are available for a total of three samples. The results of these toxicity tests demonstrated that none of the pore-water samples from the Clooney Island Loop were toxic to aquatic plants (Figure D-6; Table D-20). Overall, COPCs in pore water are considered to pose a low risk to aquatic plants in this reach of the UCR AOC.

Contraband Bayou Reach - Based on the results of the pore-water toxicity tests, it is apparent that COPCs generally pose an indeterminate risk to aquatic plants in the Contraband Bayou reach of UCR (Table D-20). Of the three pore-water samples tested for this reach, one was toxic to the alga, *Ulva fasciata*. This sample was collected on the south side of the bayou in the vicinity of Charvais Drive (Figure D-6). Overall, COPCs in pore water are considered to pose an indeterminate risk to aquatic plants in this reach of the UCR AOC.

Coon Island Loop Reach - As is the case for the other reaches in the UCR AOC, exposure to pore-water samples from the Coon Island Loop generally poses a low risk to aquatic plants. Of the five samples that were tested from this reach, one was found to be toxic to the alga, *Ulva fasciata* (Table D-20). This sample was collected from the southeast portion of the Coon Island Loop (Figure D-6). Overall, COPCs in pore water are considered to pose a low risk to aquatic plants in this reach of the UCR AOC.

In summary, pore water from sediments within the UCR AOC are generally of sufficient quality to support the normal survival, growth, and reproduction of aquatic plants. The incidence and magnitude of toxicity to the alga, *Ulva fasciata,* were similar to those that were observed for reference areas. Samples of concern with respect to effects on aquatic plants were obtained from the eastern portion of Lake Charles, Contraband Bayou roughly 1 km from the mouth, and the southeastern portion of the Coon Island Loop.

3.6.1.4 Contaminants of Concern in the Upper Calcasieu River Area of Concern

Following the assessment of risks to the aquatic plant community, it is important to identify the factors that are causing or substantially contributing to adverse effects on aquatic plants. In this document, the substances that occur in surface water or pore water from UCR AOC sediments at concentrations that are sufficient to cause or substantially contribute adverse effects on the survival, growth, or reproduction of aquatic plants are termed contaminants of concern (COCs). The COCs in the UCR AOC, relative to the potential for adversely affecting aquatic plant communities, were identified by comparing the concentrations of COPCs in surface water or pore water to the concentrations of those substances in surface water or pore water from reference areas and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for each biological endpoint were examined, when possible, to confirm that there was concordance between chemical concentrations and biological responses.

Based on the results of the exposure assessment, chromium (total and dissolved), lead (dissolved), and nickel (total and dissolved) occurred in surface water from the UCR

AOC at levels a factor of two or more higher than the 95% UCL for reference areas (Table D-12). Subsequent screening against benchmarks for surface-water chemistry revealed that total and dissolved nickel represent preliminary COCs relative to the aquatic plant community (Table D-22). No toxicity tests were conducted on surface waters from Calcasieu Estuary; therefore, it was not possible to evaluate the degree of concordance between COPC concentrations and biological effects. These results indicate that total and dissolved nickel are the primary COCs in surface water from the UCR-AOC (Table D-23). Historic and/or ongoing sources of these substances are known to exist in the estuary (see BPF for more information on the sources of these COCs). Insufficient information was available to determine if many other substances represent surface-water COCs, including: hydrogen sulfide; methyl mercury; various individual PAHs; various PCB mixtures (Aroclors); total PCBs; HCBD; various chlorinated benzenes; and, carbon disulfide.

Data on the concentrations of COPCs in pore-water samples also provides important information for identifying COCs. Comparison of the measured concentrations of COPCs in pore water to the concentrations in pore water from reference sediments (Table D-13), to the toxicity thresholds for pore water (Table D-24), and in the effects and no effects distributions (based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*; Table D-25) indicates that none of the COPCs considered should be identified as COCs in pore water (Table D-26). It should be noted, however, that insufficient data were available to determine if the following substances represent COCs: chromium (total and dissolved); mercury (total and dissolved); methyl mercury; BEHP; various chlorinated benzenes; chlorinated ethanes; acetone; and, carbon disulfide (Table D-26).

The substances that occur in surface-water or pore-water samples at concentrations above those in reference areas, above the selected benchmarks, and show concordance with the biological response data, represent the COCs relative to effects on aquatic plants. In the UCR AOC, the COCs include total and dissolved nickel (Table D-23 and D-26).

3.6.2 Bayou d'Inde Area of Concern

Bayou d'Inde is one of the major tributaries to the Calcasieu River (Figure D-1). From its headwaters near Sulphur, Louisiana, Bayou d'Inde flows in a southeasterly direction some 16 km to its confluence with the Calcasieu Ship Channel (or roughly 11 km from the I-10 bridge to the mouth). Over that distance, Bayou d'Inde is joined by several tributaries, the largest of which is Maple Fork. The lower portions of the bayou are characterized by hydraulic connections (i.e., channels that connect the wetlands to the bayou) with a great deal of off-channel wetland habitat, the largest of which is the Lockport Marsh. The areas of interest within the BI AOC include Lower Bayou d'Inde and Middle Bayou d'Inde (MacDonald *et al.* 2001). To facilitate assessment of risks to the aquatic plant community, the BI AOC was divided into five reaches, including:

- Upper Bayou d'Inde (i.e., from the headwaters to the Highway 108 bridge; Figure D-3);
- Middle Bayou d'Inde (i.e., from the Highway 108 bridge to the confluence with PPG Canal; Figure D-3);
- Lower Bayou d'Inde (i.e., the mainstem from the confluence with PPG Canal to the confluence with the Calcasieu River; Figure D-3);

- PPG Canal (i.e., from the headwaters to the confluence with Bayou d'Inde; Figure D-3); and,
- Lockport Marsh (i.e., the wetland areas located near the mouth of Bayou d'Inde, but excluding the Lower Bayou d'Inde mainstem; Figure D-3).

The risks to aquatic plant community posed by exposure to surface water or pore water were evaluated for each of these reaches and for the BI AOC as a whole. Additionally, hot spots with respect to surface-water or pore-water contamination were identified when possible.

3.6.2.1 Nature of Effects on the Aquatic Plant Community in the Bayou d'Inde Area Of Concern

In total, data on three measurement endpoints were used to determine if adverse effects on the aquatic plant community were occurring in the BI AOC in response to exposure to COPCs, including surface-water chemistry, pore-water chemistry, and pore-water toxicity. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to aquatic plant communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring or are likely to be occurring within the study area, and to determine the nature of those effects.

The surface-water chemistry data collected during the Phase I RI were evaluated using chronic toxicity thresholds for aquatic plants (or equivalent benchmarks). Two (29%) of the surface-water samples (n=7) collected within this AOC had levels of hydrogen sulfide or ammonia sufficient to adversely affect aquatic plants (Table D-14). Based on the predicted incidence of toxicity, the levels of metals in surface water from the UCR AOC do not pose incremental risks to aquatic plants (Table D-15). Similarly, the predicted incidence of toxicity to aquatic plants is similar in the UCR AOC (19%; n=21) and in reference areas (0%; n=1), based on the measured concentrations of organic substances in surface water (Table D-16). Overall, the results of this assessment indicate that exposure to surface water poses risks to the aquatic plant community (i.e., survival, growth, or reproduction) in portions of the BI AOC.

The pore-water chemistry data collected during the Phase II RI were compared to the chronic toxicity thresholds (Tables D-17 to D-19). The results of this evaluation indicate that one or more chronic toxicity thresholds for metals were exceeded in all of the pore-water samples (n=15) collected from the BI AOC; the predicted incidence of pore-water toxicity was also 100% for the samples from the reference areas (Table D-18; based on one or more exceedances of the toxicity thresholds for metals in pore water). However, when only the concentrations of hydrogen sulfide and ammonia were considered, the predicted incidence of pore-water toxicity in the BI AOC was 87% (n=31; compared to 21% for the reference areas; Table D-17). The concentrations of organic substances in pore water were not sufficient to pose incremental risks to aquatic plants in the BI AOC, however (Table D-19). Nevertheless, these results suggest that the concentrations of certain COPCs in pore water from BI AOC sediments are sufficient to cause chronic toxicity to sensitive aquatic plant species and, as a result, adversely affect the aquatic plant community.

The results of pore-water toxicity tests provide a basis for assessing the risks to aquatic plant communities associated with exposure to COPCs in the Calcasieu

Estuary. Overall, four of the 15 pore-water samples (27%) from the BI AOC were toxic to the alga, *Ulva fasciata,* in 96-h toxicity tests when zoospore germination or growth were considered (Table D-20; Figure D-8). By comparison, the incidence of toxicity was 14% (1 of 7 samples) for algal zoospores exposed to pore water from sediments from reference areas. While these data suggest that sediment quality conditions sufficient to adversely affect the survival, growth, or reproduction of aquatic plants do not occur throughout the BI AOC, such effects are likely to occur in certain locations within the AOC.

When considered together, these three lines of evidence indicate that exposure to surface water or pore water may be posing significant risks to the aquatic plant community (i.e., the survival, growth, or reproduction of aquatic plants in the BI AOC may be adversely affected). Therefore, it is concluded that significant effects on the aquatic plant community are likely occurring in the BI AOC. The apparent disagreement between the results of the assessments based on pore-water chemistry and pore-water toxicity may reflect the fact that pore-water samples were frozen and thawed prior to initiating the toxicity tests with the alga, *Ulva fasciata*. Hence, the levels of hydrogen sulfide and/or ammonia were likely lower during the toxicity tests than was the case in unfrozen pore-water samples.

3.6.2.2 Magnitude of Effects on the Aquatic Plant Community in the Bayou d'Inde Area of Concern

The magnitude of the effects on aquatic plants in the BI AOC was evaluated using one line of evidence: pore-water toxicity. Based on the results of 96-h pore-water toxicity tests with alga, *Ulva fasciata*, (observed magnitude of toxicity), it is apparent that exposure to pore water from the BI AOC sediments generally does not adversely affect the germination of algal zoospores. Of the 15 pore-water samples that were collected from the BI AOC, a total of 11 (73%) were found to pose a low risk to algae (i.e., germination rates of algal zoospores were similar to those observed for samples from reference areas; Table D-21). By comparison, the germination of algal zoospores was reduced by >20% in four of the samples (27%) from the BI AOC (Table D-21; Figure D-9).

The information on the observed magnitude of toxicity to the alga, *Ulva fasciata,* indicates that exposure to pore water from the BI AOC sediments generally poses a low risk to the aquatic plant community (Table D-21). Nevertheless, these results demonstrate that exposure to COPCs and associated toxicity pose unacceptable risks to aquatic plant communities at various locations within this AOC.

3.6.2.3 Preliminary Assessment of the Areal Extent of Effects on the Aquatic Plant Community in the Bayou d'Inde Area of Concern

A preliminary assessment of the areal extent of adverse effects on aquatic plant communities in the BI AOC was conducted using the pore-water toxicity data that were collected in Phase II of the RI. To support this evaluation of the spatial distribution of contamination, each sample was classified into one of three categories (i.e., low, indeterminate, or high), based on the risk that it posed to aquatic plants (as indicated by the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*). Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial. The reaches that were considered in this analysis included Upper Bayou d'Inde, Middle Bayou d'Inde, Lower Bayou d'Inde mainstem, Lockport Marsh, and PPG Canal.

Upper Bayou d'Inde Reach - Pore-water toxicity data are available for a total of two samples from the Upper Bayou d'Inde reach of the BI AOC. The results of these toxicity tests indicate that a pore-water sample from Upper Bayou d'Inde immediately south of the Highway I-10 bridge was toxic to aquatic plants (Figure D-8; Table D-20). Overall, COPCs in pore water are considered to pose an indeterminate risk to aquatic plants in this reach of the BI AOC.

Middle Bayou d'Inde Reach - For the Middle Bayou d'Inde reach of the BI AOC, pore-water toxicity data are available for a total of three samples. The results of these toxicity tests indicate that the sample from a station located in Maple Fork was toxic to the alga, *Ulva fasciata* (Figure D-8; Table D-20). Overall, COPCs in pore water are considered to pose an indeterminate risk to aquatic plants in this reach of the BI AOC.

Lower Bayou d'Inde Reach - Based on the results of the pore-water toxicity tests, it is apparent that COPCs generally pose a low risk to aquatic plants in the mainstem portion of Lower Bayou d'Inde. None of the samples (n=2) collected from this reach were found to be toxic to the alga, *Ulva fasciata* (Figure D-8; Table D-20). Overall, COPCs in pore water are considered to pose a low risk to aquatic plants in this reach of the BI AOC.

Lockport Marsh Reach - A total of six pore-water samples were collected from the Lockport Marsh portion of Lower Bayou d'Inde. Of these, two pore-water samples were found to be toxic to aquatic plants (i.e., based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata)*. Both samples were collected in the central portion of Lockport Marsh (Figure D-8; Table D-20).

These results indicate that COPCs in pore water generally pose an indeterminate risk to aquatic plants in the Lockport Marsh reach of the BI AOC.

PPG Canal Reach - Two pore-water samples were collected from PPG Canal reach of the BI AOC. The results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*, indicate that neither of these samples were toxic to aquatic plants (Figure D-6). As such, COPCs in pore water are considered to pose a low risk to aquatic plants in this reach of the BI AOC.

In summary, pore water from sediments within the BI AOC generally pose variable risks to aquatic plant communities. The incidence of toxicity to the alga, *Ulva fasciata*, was similar (i.e., 27%; n=15) to the incidence of toxicity that was observed for reference areas (i.e., 14%; n=7). Importantly, a number of hot spots with respect to sediment contamination were identified within the BI AOC, with the highest risks to aquatic plants occurring in the portion of Upper Bayou d'Inde near the I-10 bridge, Maple Fork, and Lockport Marsh. As such, the survival, growth, and reproduction of aquatic plants are likely to be impaired in portions of the BI AOC.

3.6.2.4 Contaminants of Concern in the Bayou d'Inde Area of Concern

Following the assessment of risks to the aquatic plant community, it is important to identify the factors that are causing or substantially contributing to adverse effects on aquatic plants. In this document, the substances that occur in surface water or pore water from the BI AOC at concentrations that are sufficient to cause or substantially contribute adverse effects on the survival, growth, or reproduction of aquatic plants are termed contaminants of concern (COCs). The COCs in the BI AOC, relative to the potential for adversely affecting aquatic plant communities, were identified by comparing the concentrations of COPCs in surface water or pore water to the concentrations of those substances in surface water or pore water from reference areas and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for each biological endpoint were examined, when possible, to confirm that there was concordance between chemical concentrations and biological responses.

Based on the results of the exposure assessment, a total of eight substances or groups of substances occurred in surface water from the BI AOC at levels a factor of two or more above the 95% UCL for reference areas (Table D-12). Subsequent screening against benchmarks for surface-water chemistry revealed that four of these analytes, total ammonia, dissolved copper, and total and dissolved nickel, represent COCs relative to the aquatic plant community (Tables D-22 and D-27). As no toxicity tests were conducted on surface water samples from the BI AOC, it was not possible to evaluate the degree of concordance between COPC concentrations and biological effects. Historic and/or ongoing sources of these substances are known to exist in the estuary (see BPF for more information on the sources of these COCs). Insufficient information was available to determine if many other substances represented COCs, including hydrogen sulfide, methyl mercury, various individual PAHs, various PCB mixtures (i.e., Aroclors), BEHP, and various chlorinated benzenes.

Data on the concentrations of COPCs in pore-water samples also provides important information for identifying COCs. Comparison of the measured concentrations of COPCs in pore water to the concentrations in pore water from reference sediments (Table D-13), to the toxicity thresholds for pore water (Table D-24), and in the effects and no effects distributions (based on the results of 96-h pore-water toxicity tests with the alga, *Ulva fasciata*; Table D-25) indicates that none of the COPCs considered should be identified as COCs in pore water (Table D-28). It should be noted, however, that insufficient data were available to determine if the following substances represent COCs: chromium (total and dissolved); mercury (total and dissolved); methyl mercury; 1-methylphenanthrene; BEHP; various chlorinated benzenes; chlorinated ethanes; acetone; and, carbon disulfide (Table D-26).

The substances that occur in surface-water or pore-water samples at concentrations above those in reference areas, above the selected benchmarks, and show concordance with the biological response data represent the COCs relative to effects on aquatic plants. In the BI AOC, the COCs in surface water or pore water include ammonia, dissolved copper, and total and dissolved nickel.

3.6.3 Middle Calcasieu River Area of Concern

The Middle Calcasieu River comprises the portion of the watershed from the Highway 210 bridge to the outlet of Moss Lake (a distance of roughly 12 km), excluding Bayou d'Inde (Figures D-4a and D-4b). The primary physiographic features in this portion of the study area include the Calcasieu Ship Channel, Prien Lake, the original Calcasieu River channel, and Moss Lake. For this assessment, the Indian Wells Lagoon and Bayou Olsen were also included in the Middle Calcasieu River study area. The areas of interest within the MCR AOC include south Prien Lake and the Indian Wells Lagoon outflow (MacDonald *et al.* 2001). To facilitate assessment of risks to the aquatic plant community, the MCR AOC was divided into five reaches, including:

- Middle Calcasieu River - Mainstem (i.e., Calcasieu Ship Channel and the old river channel, to the outlet of Moss Lake, excluding the portion of the channel south of Prien Lake; Figures D-4a and D-4b);
- Prien Lake and the upper old river channel (Figure D-4a);
- Indian Wells Lagoon (Figure D-4a);
- Bayou Olsen (i.e., from the headwaters to the mouth; Figure D-4b); and,
- Moss Lake (i.e., excluding the Calcasieu Ship Channel; Figure D-4b).

The risks to aquatic plant community posed by exposure to surface water or pore water were evaluated for each of these reaches and for the UCR AOC as a whole. Additionally, hot spots with respect to contaminated surface water and pore water were identified when possible.

3.6.3.1 Nature of Effects on the Aquatic Plant Community in the Middle Calcasieu River Area of Concern

In total, data on three measurement endpoints were used to determine if adverse effects on the aquatic plant community were occurring in the MCR AOC in response to exposure to COPCs, including surface-water chemistry, pore-water chemistry, and pore-water toxicity. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to aquatic plant communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring or are likely to be occurring within the study area, and to determine the nature of those effects.

The surface-water chemistry data collected during the Phase I RI were evaluated using chronic toxicity thresholds for aquatic plants (or equivalent benchmarks). None of the surface-water samples (n=4) collected within the AOC had levels of hydrogen sulfide or ammonia sufficient to adversely affect aquatic plants (Table D-14). Based on the predicted incidence of toxicity, the levels of metals in surface water samples (n=22) from the MCR AOC do not pose incremental risks to aquatic plants (Table D-15). Similarly, the predicted incidence of toxicity to aquatic plants is similar in the MCR AOC (5%; n=22) and in reference areas (0%; n=1), based on the measured concentrations of organic substances in surface water (Table D-16). Therefore, it is unlikely that exposure to surface water poses significant risks to aquatic plant community (i.e., survival, growth, and reproduction are unlikely to be adversely affected).

The pore-water chemistry data collected during the Phase II RI were also compared to the chronic toxicity thresholds (Tables D-17 to D-19). The results of this evaluation indicate that the chronic toxicity thresholds for total ammonia or hydrogen sulfide were exceeded in 47% of the samples (i.e., 7 of 15) that were collected from the MCR AOC (based on one or more exceedances of the toxicity thresholds for pore water; Table D-17). For the metals, one or more chronic toxicity thresholds were exceeded in all of the pore-water samples (n=8) collected from the MCR AOC of the Calcasieu Estuary (Table D-18), which is the same incidence of toxicity that was predicted for the reference areas. By comparison, 25% of the pore-water samples (n=8) from the MCR AOC had levels of organic substances that were sufficient to adversely affect aquatic plants (Table D-19). These data suggest that the concentrations of COPCs in pore water from Calcasieu Estuary sediments are sufficient to cause chronic toxicity to sensitive aquatic plants and, as a result, adversely affect the aquatic plant community.

The results of pore-water toxicity tests provide a basis for assessing the risks to aquatic plant communities associated with exposure to COPCs in the Calcasieu Estuary. Overall, one of the eight pore-water samples (13%) from the MCR AOC were toxic to the alga, *Ulva fasciata*, in 96-h toxicity tests when zoospores germination or growth were considered (Figures D-10a and D-10b; Table D-20). The incidence of toxicity was 14% (n=7) for algal zoospores exposed to pore water from sediments from reference areas. These data suggest that sediment quality conditions (i.e., as reflected by pore-water toxicity) sufficient to adversely affect the survival, growth, or reproduction of aquatic plants occur only infrequently in the MCR AOC.

When considered together, these three lines of evidence indicate that exposure to surface water or pore water is likely to pose significant risks to the aquatic plant community only infrequently in the MCR AOC (i.e., the survival, growth, or reproduction of aquatic plants may be adversely affected by exposure to COPCs in pore water). Therefore, it is concluded that significant effects on the aquatic plant community are occurring at certain locations in the MCR AOC.

3.6.3.2 Magnitude of Effects on the Aquatic Plant Community in the Middle Calcasieu River Area of Concern

The magnitude of the effects on aquatic plants in the MCR AOC was evaluated using one line of evidence: pore-water toxicity. Based on the results of 96-h toxicity tests with the alga, *Ulva fasciata* (observed magnitude of toxicity), it is apparent that exposure to pore water from the MCR AOC sediments generally does not adversely affect the germination of algal zoospores. Of the eight pore-water samples that were collected from the MCR AOCs, seven (88%) were found to pose a low risk to algae (i.e., germination rates of algal zoospores were similar to those observed for samples from reference areas; Figures D-11a and D-11b; Table D-21). By comparison, the germination of algal zoospores was reduced by >20% in one of the samples (13%) from the MCR AOC.

The information on the observed magnitude of toxicity to the alga, *Ulva fasciata,* indicates that exposure to pore water from the MCR AOC sediments generally poses a low risk to the aquatic plant community (Table D-21). Nevertheless, these results demonstrate that conditions sufficient to adversely affect algal zoospores germination do exist within the MCR AOC.

3.6.3.3 Preliminary Assessment of the Areal Extent of Effects on the Aquatic Plant Community in the Middle Calcasieu River Area of Concern

A preliminary assessment of the areal extent of adverse effects on aquatic plant communities in the MCR AOC was conducted using the pore-water toxicity data that were collected in the Phase II RI. To support this evaluation of the spatial distribution of contamination, each sample was classified into one of three categories (i.e., low, indeterminate, or high), based on the risk that it posed to aquatic plants (as indicated by the results of 96-h toxicity tests with the alga, *Ulva fasciata)*. Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial Analyst software. The reaches that were considered in this analysis included the Middle Calcasieu River mainstem, Prien Lake and the upper old river channel, Indian Wells Lagoon, Bayou Olsen, and Moss Lake.

Middle Calcasieu River - Mainstem Reach - No pore-water toxicity data are available from the Middle Calcasieu River mainstem reach of the MCR AOC.

Therefore, it was not possible to evaluate the areal extent of adverse effects on the aquatic plant community in this reach of the MCR AOC.

Prien Lake and Upper Old River Channel Reach - For the Prien Lake and upper old river channel reach of the MCR AOC, pore-water toxicity data are available for a total of two samples (Table D-20). The results of these toxicity tests indicate that none of the samples from this reach of the MCR AOC were toxic to the alga, *Ulva fasciata* (Figure D-10a). Therefore, COPCs in pore water are considered to pose a low risk to aquatic plants in this reach of the MCR AOC.

Indian Wells Lagoon Reach - Based on the results of pore-water toxicity tests, it is apparent that COPCs pose an indeterminate risk to aquatic plants in the Indian Wells Lagoon reach of Middle Calcasieu River (Table D-20). One of the samples (n=2) collected from Indian Wells Lagoon was found to be toxic to the alga, *Ulva fasciata* (Figure D-10a). None of the zoospores exposed to pore water from sediments collected from the more northerly of the two stations sampled germinated. Therefore, COPCs in pore water are considered to pose an indeterminate risk to aquatic plants in this reach of the MCR AOC.

Bayou Olsen Reach - Based on the available pore-water toxicity data, risks to the aquatic plant community were classified as low within the Bayou Olsen reach of the MCR AOC (Table D-20). Neither of the two pore-water samples from Bayou Olsen were toxic to the alga, *Ulva fasciata* (Figure D-10b). Therefore, COPCs in pore water are considered to pose a low risk to the aquatic plant community.

Moss Lake Reach - For Moss Lake, pore-water toxicity data are available for two samples. Neither of these pore-water samples were found to be toxic to the alga, *Ulva fasciata* (Figure D-10b; Table D-20). Therefore, COPCs in pore water are considered to pose a low risk to the aquatic plant community in the Moss Lake reach of the MCR AOC.

In summary, exposure to pore water from sediments within the MCR AOC generally poses low risks to aquatic plant communities. The incidence of toxicity (13%; n=8) to the alga, *Ulva fasciata*, was similar to the incidence of toxicity that was observed for reference areas (14%; n=7). However, conditions in one of the pore-water samples (13%) from the reach were sufficient to pose a significant risk to aquatic plants. The hot spot with respect to pore-water toxicity was the northern portion of the Indian Wells Lagoon reach of the MCR AOC.

3.6.3.4 Contaminants of Concern in the Middle Calcasieu River Area of Concern

Following the assessment of risks to the aquatic plant community, it is important to identify the factors that are causing or substantially contributing to adverse effects on aquatic plants. In this document, the substances that occur in surface water or pore water from the MCR AOC at concentrations that are sufficient to cause or substantially contribute adverse effects on the survival, growth, or reproduction of aquatic plants are termed contaminants of concern (COCs). The COCs in the MCR AOC, relative to the potential for adversely affecting aquatic plant communities, were identified by comparing the concentrations of COPCs in surface water and pore water to the concentrations of those substances in surface water or pore water from reference areas and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for each biological endpoint were examined, when possible, to confirm that there was concordance between chemical concentrations and biological responses.

Based on the results of the exposure assessment, chromium (total and dissolved), mercury (total), and nickel (total and dissolved) occurred in surface water from the MCR AOC at levels a factor of two or more above the 95% UCL for reference areas (Table D-12). Subsequent screening against benchmarks for surface-water chemistry revealed that of these substances, nickel (total and dissolved) occurred in surface water at levels that could adversely affect the aquatic plant community (Tables D-22 and D-29). As no toxicity tests were conducted on surface-water samples from the MCR AOC, it was not possible to evaluate the degree of concordance between COPC concentrations and biological effects. Therefore, total and dissolved nickel represent the principal COCs in the surface water in the MCR AOC. Historic and/or ongoing sources of this substance are known to exist the estuary (see BPF for more information on the sources of these COCs). Insufficient information was available to determine if many other substances represented COCs, including: hydrogen sulfide; methyl mercury; various individual PAHs; various PCB mixtures (i.e., Aroclors); total PCBs; various chlorinated benzenes; and, carbon disulfide.

Data on the concentrations of COPCs in pore-water samples also provide important information for identifying COCs. Comparison of the measured concentrations of COPCs in pore water to the concentrations in pore water from reference sediments (Table D-13), to the toxicity thresholds for pore water, (Table D-24) and in the effects and no effects distributions (based on the results of the *Ulva fasciata* toxicity test; Table D-25) indicates that benz(a)anthracene should be considered to be a COC in pore water (Table D-30).

The substances that occur in pore-water samples at concentrations above those in reference areas, above the selected benchmarks, and show concordance with the biological response data, represent the COCs relative to effects on aquatic plants. In the MCR AOC, the COCs include total and dissolved nickel and benz(a)anthracene.

4.0 Uncertainty Analysis

There are a number of sources of uncertainty in assessments of risk to the aquatic plant community, including uncertainties in the conceptual model, in the exposure assessment, and in the effects assessment. As each of these sources of uncertainty can influence the estimations of risk, it is important to describe and, when possible, quantify the magnitude and direction of such uncertainties. The purpose of this section is to evaluate uncertainty in a manner that facilitates attribution of the level of confidence that can be placed in the assessments conducted using the various lines of evidence. Accordingly, the uncertainties associated with the assessment of risks to aquatic plant communities are described in the following sections.

4.1 Uncertainties Associated with the Conceptual Model

The conceptual model is intended to define the linkages between stressors, potential exposure, and predicted effects on ecological receptors. As such, the conceptual model provides the scientific basis for selecting assessment and measurement endpoints to support the risk assessment process. Potential uncertainties arise from lack of knowledge regarding ecosystem functions, failure to adequately address spatial and temporal variability in the evaluations of sources, fate, and effects, omission of stressors, and overlooking secondary effects (USEPA 1998). The types of uncertainties that are associated with the conceptual model that links contaminant sources to effects on the aquatic plant community include those associated with the identification of COPCs, environmental fate and transport of COPCs, exposure pathways, receptors at risk, and ecological effects. Of these, the identification of exposure pathways probably represents the primary source of uncertainty in the conceptual model.

In this assessment, it was assumed that exposure to surface water and pore water represents the most important pathways for exposing aquatic plant communities to COPCs. While all aquatic plants are exposed to surface water, phytoplankton and periphyton are less likely to be exposed to pore water. Because COPC concentrations tend to be higher in pore water than they are in surface water, consideration of exposure to pore water could result in over-estimation of risks to certain groups of aquatic plants. Nevertheless, it is reasonable to consider this exposure route for rooted aquatic plants, which tend to dominate the wetland areas throughout the estuary.

4.2 Uncertainties Associated with the Exposure Assessment

The exposure assessment is intended to describe the actual or potential co-occurrence of stressors with receptors. As such, the exposure assessment identifies the exposure pathways and the intensity and extent of contact with stressors for each receptor or group of receptors at risk. There are a number of potential sources of uncertainty in the exposure assessment, including measurement errors, extrapolation errors, and data gaps.

In this assessment, two types of measurements were used to evaluate exposure of aquatic plant communities to COPCs, including chemical analyses of environmental media (i.e., surface water and pore water) and toxicity tests conducted using indicator species. Relative to the pore-water and surface-water chemistry data, analytical errors and descriptive errors represent potential sources of uncertainty. Three approaches were used to address concerns relative to these sources of uncertainty. First, analytical errors were be evaluated using information on the accuracy, precision, and detection limits (DL) that are generated to support the Phase I and Phase II sampling programs. The results of this analysis indicated that most of the data used in this assessment met the project data quality objectives (see Appendix B1 for more details). Second, all data entry, data translation, and data manipulations were audited to assure their accuracy. Data auditing involved 10% number-for-number checks against the primary data source initially, increasing to 100% number-for-number checks if significant errors were detected in the initial auditing step. Finally, statistical analyses of resultant data were conducted to evaluate data distributions, identify the appropriate summary statistics to generate, and evaluate the variability in the observations. As such, measurement errors in the pore-water and surface-water chemistry data are considered to be of minor importance and are unlikely to substantially influence the results of the risk assessment.

The treatment of surface-water or pore-water chemistry data has the potential to influence the results of the BERA. In particular, the treatment of less than detection limit data can effect the results of the exposure assessment and the risk characterization. A number of investigators have evaluated the implications of applying various procedures for estimating the concentrations of COPCs from less than detection limit data (Gaskin *et al.* 1990; Porter and Ward 1991; El-Shaawari and Esterby 1992; Clarke and Brandon 1994). While there is no consensus on which data censoring method should be used in various applications, the simplest methods tend to be used most frequently, including deletion of non-detect values or substitution of a constant, such as zero, the detection limit, or one-half the detection limit (USACE 1995).

To address the need for guidelines for statistical treatment of less than detection limit data, the United States Army Corps of Engineers (USACE 1995) conducted a simulation study to assess the performance of 10 methods for censoring data. The results of that investigation indicated that no single data censoring methods works best in all situations. Accordingly, USACE recommended a variety of methods depending on the proportion of the data that requires censoring, the distribution and variance of the data, and the type of data transformation. For data sets for which a low to indeterminate proportion of the data require censoring, substitution of the detection limit is generally the preferred methods (i.e., to optimize statistical power and control type I error rates). However, as the proportion of the data that requires censoring and the coefficient of variation of the data increase, statistical power is better maintained by substituting of one-half the detection for the less than detection limit data, particularly for lognormally distributed and transformed data. Substitution of zero or other constants was also recommended for a variety of circumstances. Overall, it was concluded that simple substitution methods work best to maintain power and control error rates in statistical comparisons of chemical concentration data (USACE 1995).

In this analysis, decisions regarding the treatment of less than detection limit data were made by considering the recommendations that have emerged from previous investigations in the context of their potential effects on the results of the BERA. Including all of the surface-water or pore-water chemistry data that were collected in the Calcasieu Estuary RI, roughly 80% of the data required censoring prior to data analysis. To minimize the potential effects of the less than detection limit data on the results of the BERA, none of the less than detection limit data for which the detection limits were greater than the corresponding toxicity thresholds for surface-water or pore-water chemistry (i.e., benchmarks) was used in the exposure analysis. Consistent with the guidance developed by USACE (1995), one-half of the detection limit was substituted for all of the other less than detection limit data. This procedure facilitated the estimation of distributions of the concentrations of COPCs and eliminated the potential for identifying significant risks based on less than detection limit data.

Selection of an alternate procedure for treating the less than detection limit data has the potential for influencing the results of the BERA. For example, substitution of zero for less than detection limit data would have altered the distributions of the COPC concentration data for the three AOCs and the reference area (i.e., the estimated 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentile concentrations would likely have been lower than the estimates developed for the exposure assessment). Likewise, substitution of the detection limit for the less than detection limit data would have altered the distributions of the COPC concentration data for the three AOCs and the reference area (i.e., the estimated 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentile concentrations would likely have been higher than the estimates developed for the exposure assessment). Although the influence of these alternate methods on the estimate of the 75th or 95th percentile concentration would likely have been relatively minor, their selection could have influenced the identification of COCs for one or more AOCs. However, neither the nature, magnitude, nor areal distribution of risks to the aquatic plant communities was affected by the selection of data treatment methods. As such, the potential impact of the methods that were selected for treating less than detection limit data on the results of the BERA are considered to be minor.

Data gaps also represent a source of uncertainty in the assessments of exposure for aquatic receptors. For example, the available data on the chemical composition and toxicity of pore waters were limited to 45 samples. As such, the areal distribution of pore-water samples is much more limited than was the case for whole-sediment samples. Although somewhat more data was available on surface-water chemistry (i.e., up to 56 samples) than was the case for pore-water chemistry, data gaps represent a more serious concern for the surface-water data. Some of the issues that need to be considered in the gap analysis for surface water include incomplete data for reference areas, missing analytes, elevated detection limits (relative to benchmarks; e.g., PCBs, chlorinated benzenes), incomplete spatial coverage of the study area, and incomplete information on temporal variability of surface-water quality. These limitations make it difficult to assess exposure of aquatic plant communities to COPCs in the Calcasieu Estuary.

4.3 Uncertainties in the Effects Assessment

The effects assessment is intended to describe the effects that are caused by stressors, link them to the assessment endpoints, and evaluate how effects change with fluctuations in the levels (i.e., concentrations) of the various stressors. There are several potential sources of uncertainty in the assessment of effects on aquatic receptors, including measurement errors, extrapolation errors, and data gaps.

In this investigation, the effects on aquatic plant communities associated with exposure to COPCs were evaluated using several types of information, including toxicity benchmarks for surface-water chemistry, toxicity benchmarks for pore water, and pore-water toxicity tests. Although the toxicity benchmarks are not subject to measurement errors, the toxicity tests are. For this reason, potential measurement errors associated with toxicity tests were evaluated in the uncertainty analysis. More specifically, the data on negative controls and positive controls were examined to identify potential measurement errors. In addition, the results obtained from samples collected in the reference areas were considered in this analysis. More specifically, a reference envelope approach was used to classify pore-water samples as toxic or not toxic. Because this approach facilitated the determination of the normal range of responses for samples from reference areas and only samples for which the response was beyond the 95% lower confidence limit (LCL) (i.e., 25^{th} percentile) were designated as toxic, the probability of incorrectly classifying a not toxic sample as toxic is roughly 0.025. However, the probability of incorrectly classifying a toxic sample as not toxic is probably higher. Therefore, application of the reference envelope approach may tend to under-estimate risks to the aquatic plant community.

There are several sources of extrapolation errors in the effects assessment for the Calcasieu Estuary BERA. First, the toxicity benchmarks for surface water and pore water were primarily the concentrations that corresponded to chronic toxicity thresholds for freshwater plants. While these benchmarks are intended to be broadly applicable to freshwater systems, their relevance to salt marsh plants could be questioned. Second, indicator species have been used in this investigation to assess the effects of contaminated pore water on the aquatic plant community. Uncertainties associated with the application of this approach are considered to be minor as sea lettuce (*Ulva fasciata*) is considered to be an appropriate surrogate for both freshwater and estuarine aquatic plant species (Hooten and Carr 1998).

Uncertainty in the effects assessment for aquatic receptors is also increased by data gaps. To the extent possible, this source of uncertainty was mitigated by collecting detailed information on the effects of COPCs in the Calcasieu Estuary. In addition, the use of multiple lines of evidence provides a basis for minimizing the influence of data gaps on the effects assessment. Nevertheless, limitations on certain types of data, such as concentration-response data for various aquatic plant species, makes it difficult to fully evaluate the effects of COPC exposures on the aquatic plant communities. For this reason, the present assessment could over-estimate or under-estimate risks to the aquatic plant community.

5.0 Integrated Assessment of Risks to Aquatic Plants in the Calcasieu Estuary Using a Weight of Evidence Approach

Information on three lines of evidence was compiled to support the assessment of risks to the aquatic plant community associated with exposure to surface water or pore water in the Calcasieu Estuary. The previous sections of this appendix present the information of each of these individual lines of evidence and interpret that information to evaluate effects on the survival, growth, or reproduction of aquatic plants. As such, the previous evaluations were used to provide the information needed to determine if adverse effects on aquatic plants are occurring or are likely to be occurring within the Calcasieu Estuary, to evaluate the nature, severity, and areal extent of such effects, and to identify the substances that are causing or substantially contributing to effects on the aquatic plant community.

Each of the lines of evidence that was used in the assessment of risks to the aquatic plant communities has certain strengths and limitations that influence its application in the risk assessment process. For this reason, an uncertainty analysis was conducted to evaluate the level of confidence that can be placed in the results of analyses conducted using the individual lines of evidence (Section 4.0). Importantly, the uncertainty associated with the overall assessment of risks can be reduced by integrating information from multiple lines of evidence using a weight of evidence approach (Ingersoll and MacDonald 2002).

In this investigation, a simple arithmetic procedure was used to integrate information from multiple lines of evidence. In the first step of this process, the level of confidence (i.e., weight; as quantified by calculating a total evaluation score - TES; Table D-31) that could be placed in each measurement endpoint was scored from one (low) to three (high) determined based on the following considerations (i.e., adapted from Suter *et al.* 2000):

Conceptual Model:

- *Relevance of Exposure Pathway:* Evidence was given more weight if the exposure pathway examined was the most relevant for assessing the status of the assessment endpoint. For example, exposure of aquatic plants to surface water would be considered to be more relevant than exposure to whole sediments; and,
- *Relevance of the Measurement Endpoint*: Evidence was given more weight if the measurement endpoint provided a direct estimate of the status of the assessment endpoint or if validation studies have demonstrated that measurement endpoint is predictive of the assessment endpoint. For example, measurement of the survival and growth of

amphipods is considered to provide direct evidence for evaluating the survival and growth of benthic invertebrates. By comparison, data on the fertilization and development of sea urchin gametes and embryos (which are pelagic life history stages) is considered to provide less direct evidence for evaluating the reproduction of benthic invertebrates.

Exposure Assessment:

- *Level of Standardization*: Evidence was given more weight if standard methods were available and appropriately implemented to generate the data on exposure of receptors at risk to COPCs. For example, the level of standardization for surface-water chemistry and whole-sediment chemistry would be high because standard methods were used to collect, handle, transport, store, and analyse samples. By comparison, the level of standardization for pore-water chemistry would be lower because standard methods for collecting and processing samples have not been described;
- *Quality of Data:* Evidence was given more weight if the data were demonstrated to be of high quality. In this context, data quality was evaluated by considering the project data quality objectives and consider such criteria as accuracy, precision, and analytical detection limits;
- *Quantity of Data:* Evidence was given more weight if the sample size was considered to be adequate to characterize conditions within the study area;
- *Level of Temporal Coverage:* Evidence was given more weight if the data encompasses the relevant range of temporal variance of conditions. For example, a single sampling event was considered to evaluate characterize whole-sediment chemistry because such conditions are

unlikely to change substantially on a seasonal basis. In contrast, surface-water chemistry is likely to change on daily and seasonal bases, emphasizing the need for more comprehensive data sets to characterize temporal variability; and,

- *Level of Spatial Coverage:* Evidence was given more weight if the data adequately represented the geographic area that was being assessed. In this context, a measurement endpoint was scored high if samples were available from most or all of the areas of concern and associated reaches.

Effects Assessment:

- *Level of Standardization*: Evidence was given more weight if standard methods were available and appropriately implemented to generate the data on the effects associated with exposure of receptors at risk to COPCs. For example, concentration-response relationships were considered to be stronger if the underlying toxicity tests and chemical analyses were conducted using standard methods;
- *Meets Acceptability Criteria*: Evidence was given more weight if the established acceptability criteria for the measurement endpoint were met. For example, toxicity test results were weighted high if the negative and positive control results were with acceptable ranges;
- *Demonstrated Concentration - Response Relationship:* Evidence was given more weight if it demonstrated a relationship between the magnitude of exposure and the effects;
- *Relevance of the Exposure Medium*: Evidence was given more weight if the medium (i.e., surface water, sediment) considered in the assessment was consistent with the mode of exposure for the site medium. For example, the results of pore-water toxicity tests would be less relevant for

assessing the status of the aquatic plant community than would the results of surface-water toxicity tests;

- *Level of Field Validation:* Evidence was given more weight if the results of validation studies have demonstrated that the measurement endpoint provides a reliable basis estimating the status of the assessment endpoint or the status of other measurement endpoints that are predictive of the assessment endpoint.

Consideration of the result that was obtained for each measurement endpoint (e.g., observed incidence of toxicity to marine amphipods, which was score 0, 1, or 2), in conjunction with the weight (i.e., TES) that was assigned to that measurement endpoint (which was scored from 1 to 3), provided a basis for developing a risk score for each measurement endpoint and line of evidence (Section 2.5). Subsequently, a final risk score was calculated by averaging the risk score for each line of evidence.

The final risk score is intended to provide an integrated measure of the risks that exposure to surface water or pore water poses to the survival, growth, or reproduction of the aquatic plant in the Calcasieu Estuary. More specifically, the final risk score integrates the results of pore-water toxicity tests, surface-water chemical analyses, and pore-water chemical analyses. The results of the integrated assessment of risks to aquatic plants in the Calcasieu Estuary are presented in the following sections of this appendix.

5.1 Integrated Assessment of Risks to Aquatic Plants in the Upper Calcasieu River Area of Concern

Samples were collected from a total of 41 locations to support an assessment of the risks posed to the aquatic plant community associated with exposure to COPCs in surface water or pore water in the UCR AOC (Table D-32). The results of this assessment indicate that exposure to surface water and/or pore water in the UCR AOC generally posed a low risk to aquatic plants (i.e., average of the final risk scores of 1.27; n=41). Seventy-six percent of the locations (i.e., 31 of 41) within this AOC had low final risk scores (i.e., < 2; Table D-32). Nevertheless, indeterminate (2%; 1 of 41) or high (22%; 9 of 41) risks to aquatic plants were indicated for 24% of the locations (i.e., 10 of 41) from the UCR AOC (Table D-32). Consistent with the results of the preliminary analysis of the areal extent of effects, the locations where exposure to surface water or pore water posed the highest risks (i.e., relative to the survival, growth, or reproduction of aquatic plants) included the eastern and southwestern portions of the Clooney Island Loop, Clooney Island barge slip, the mouth of Bayou Verdine, the southeastern and southwestern portions of Coon Island Loop, the southern side of Contraband Bayou in the vicinity of Charvais Drive, and the southeastern portion of Lake Charles (Figure D-12). Indeterminate risks to the aquatic plant community exist in the eastern portion of Lake Charles (Figure D-12).

The biological conditions that occur within the three risk categories indicate that the samples posing indeterminate and high risks are more toxic to aquatic plants than are the samples posing low risks (Table D-33). For example, germination rates of algal zoospores averaged 88% for the samples that were designated as posing a low risk to the aquatic plant community. By comparison, alga zoospore germination averaged 60% and 37% for the samples that were designated as posing indeterminate and high risks, respectively. Therefore, it is concluded that the survival, growth, or reproduction of aquatic plants is being adversely affected by exposure to surface water or pore water in portions of the UCR AOC. Because the pore- water COCs in the UCR AOC are considered to be relatively persistent in sediments, it is likely that such effects will continue to impact the aquatic plant community unless corrective action is taken to reduce risks in high risk locations (Figure D-12).

5.2 Integrated Assessment of Risks to Aquatic Plants in the Bayou d'Inde Area of Concern

Samples were collected from a total of 52 locations to support an assessment of the risks posed to the aquatic plant community associated with exposure to COPCs in surface water and pore water in the BI AOC. The results of this assessment indicate that exposure to surface water and/or pore water in the BI AOC generally posed a low risk to aquatic plants (i.e., average of the final risk scores of 1.85; n=52). Roughly 60% of the locations (i.e., 31 of 52) within this AOC had low final risk scores (i.e., < 2; Table D-32). However, indeterminate (6%; 3 of 52) or high (35%; 18 of 52) risks to aquatic plants were indicated for 40% of the locations (i.e., 21 of 52) from the BI AOC (Table D-32). Consistent with the results of the preliminary analysis of the areal extent of effects, the locations where exposure to surface water or pore water posed the highest risks (i.e., relative to the survival, growth, or reproduction of aquatic plants) included Upper Bayou d'Inde downstream of the I-10 bridge and at the Highway 108 bridge, Middle Bayou d'Inde immediately downstream of the Highway 108 bridge and in Maple Fork, PPG Canal, and the central, northwestern, and southeastern portions of Lockport Marsh (Figure D-13). Indeterminate risks to the aquatic plant community exist at stations located in the central portion of Middle

Bayou d'Inde, in the southern portion of Lockport Marsh, and in the Lower Bayou d'Inde mainstem (Figure D-13).

The biological conditions that occur within the three risk categories indicate that the samples posing indeterminate and high risks are more toxic to aquatic plants than are the samples posing low risks (Table D-33). For example, germination rates of algal zoospores averaged 88% for the samples that were designated as posing a low risk to the aquatic plant community. By comparison, alga zoospore germination averaged 60% and 37% for the samples that were designated as posing indeterminate and high risks, respectively. Therefore, it is concluded that the survival, growth, or reproduction of aquatic plants is being adversely affected by exposure to surface water or pore water in portions of the BI AOC. Because the pore- water COCs in the BI AOC are considered to be relatively persistent in sediments, it is likely that such effects will continue to impact the aquatic plant community unless corrective action is taken to reduce risks in high risk locations (Figure D-13).

5.3 Integrated Assessment of Risks to Aquatic Plants in the Middle Calcasieu River Area of Concern

Samples were collected from a total of 37 locations to support an assessment of the risks posed to the aquatic plant community associated with exposure to COPCs in surface water or pore water in the MCR AOC. The results of this assessment indicate that exposure to surface water and/or pore water in the MCR AOC generally posed low risks to aquatic plants (i.e., average of the final risk scores of 0.69; n=37). Eighty-four percent of the locations (i.e., 31 of 37) within this AOC had low final risk scores (i.e., <2; Table D-32). Nevertheless, indeterminate (5%; 2 of 37) or high

(11%; 4 of 37) risks to aquatic plants were indicated for 16% of the samples (i.e., 6 of 37) from the MCR AOC (Table D-32). Consistent with the results of the preliminary analysis of the areal extent of effects, the locations where contaminated surface water or pore water posed the highest risks (i.e., relative to the survival, growth, or reproduction of aquatic plants) included the old river channel downstream of Prien Lake, Indian Wells Lagoon, and the west central portion of Moss Lake (Figures D-14a and D-14b). Indeterminate risks to the aquatic plant community exist in the Middle Calcasieu River upstream of Moss Lake and near the mouth of Bayou Olsen (Figure D-14b).

The biological conditions that occur within the three risk categories indicate that the samples posing indeterminate and high risks are more toxic to aquatic plants than are the samples posing low risks (Table D-33). For example, germination rates of algal zoospores averaged 88% for the samples that were designated as posing a low risk to the aquatic plant community. By comparison, alga zoospore germination averaged 60% and 37% for the samples that were designated as posing indeterminate and high risks, respectively. Therefore, it is concluded that the survival, growth, or reproduction of aquatic plants is being adversely affected by exposure to surface water or pore water in portions of the MCR AOC. Because the pore- water COCs in the MCR AOC are considered to be relatively persistent in sediments, it is likely that such effects will continue to impact the aquatic plant community unless corrective action is taken to reduce risks in high risk locations (Figures D-14a and D-14b).

6.0 Summary and Conclusions

The risks to aquatic plant communities posed by exposure to surface water and pore water were assessed in the Calcasieu Estuary. In total, information on three lines of evidence was used to determine if the survival, growth, or reproduction of aquatic plants was being adversely affected or was likely to be adversely affected by exposure to COPCs in the estuary (i.e., relative to reference conditions). The three lines of evidence that were considered in the assessment included surface-water chemistry, pore-water chemistry and pore-water toxicity.

The results of this BERA indicated that exposure to surface water and/or pore water from the Calcasieu Estuary generally posed low risks to aquatic plant communities (i.e., risks were classified as low for 72% of the 130 samples collected within the three AOCs investigated; Table D-32). However, indeterminate and high risks to the aquatic plant community were indicated for 5% (6 of 130) and 24% (31 of 130) of the samples, respectively (Table D-32). Of the three AOCs considered, the risks to the aquatic plant community were highest in Bayou d'Inde. Within this AOC, samples from the upper and lower portions of Upper Bayou d'Inde, Maple Fork, PPG Canal, and the central, northwestern, and southeastern portions of Lockport Marsh posed the highest risks (Figure D-13). Although risks to the aquatic plant community were generally lower in the UCR AOC and MCR AOC, samples posing high risk are present in the eastern and southwestern portions of Clooney Island Loop, Clooney Island barge slip, the southeastern and southwestern portions of Coon Island Loop, the mouth of Bayou Verdine, old river channel downstream of Prien Lake, west-central portion of Moss Lake, southern side of Contraband Bayou in the vicinity of Charvais Drive, southeastern portion of Lake Charles, and Indian Wells Lagoon (Table D-32; Figures D-12, D-14a, and D-14b). Risks to the aquatic plant community are generally low at the locations sampled in the reference areas, with the exception at lower Bayou Bois Connine and the central portion of Grand Bayou (Figure D-15). An assessment of the risks posed to aquatic plants associated with exposure to COPCs in the Calcasieu Estuary (i.e., on a station-by-station basis) is presented in Tables D-34 to D-52.

The results of the biological investigations conducted during the RI indicate that the magnitude of effects tends to increase with increasing risk to the aquatic plant community. For example, the germination of algal zoospores was lower in the samples that were designated as indeterminate (60±32%; n=3) and high (37±20%; n=6) risk than was the case for the low risk samples (88±7%; n=36; Table D-32). Likewise, growth rates tended to be highest for the samples that were designated as posing low risks to the aquatic plant communities (Table D-32). These results demonstrate that the survival, growth, and reproduction of aquatic plants are impaired in response to exposure to pore water at certain locations in the Calcasieu Estuary.

The results of this assessment indicated that a number of substances are causing or substantially contributing to adverse effects on the aquatic plant community in the Calcasieu Estuary. More specifically, the COCs that were considered to include:

- Total ammonia;
- Metals (dissolved copper and total and dissolved nickel); and,
- Benz(a)anthracene (Figure D-16).

All of these substances occurred in surface-water and/or pore-water samples at concentrations in excess of those observed in samples from reference areas and in excess of the selected benchmarks. In addition, the concentrations in the effects distribution were generally higher than the concentrations in the no effects distribution for one or more of the measurement endpoints in pore-water toxicity tests (e.g., germination of algal zoospores in 96-h toxicity tests). Furthermore, the results of toxicity identification evaluations conducted on pore-water samples from Calcasieu Estuary sediment samples indicate that non-polar organics and metals substantially contributed to acute toxicity to marine amphipods, *Ampelisca abdita*, in 96-h toxicity tests (SAIC 2002). Hence, the COCs identified above should be considered to be high priority for developing preliminary remediation goals.

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